UTILIZATION OF PHOSPHOKETOLASE IN THE PRODUCTION OF MEVALONATE, ISOPRENOID PRECURSORS, AND ISOPRENE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/545,083, filed October 7, 2011; the disclosure of which is hereby incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] The Sequence Listing submitted in an ASCII text file, in accordance with 37 C.F.R. § 1.821(c) and (e), is incorporated by herein by reference. The text file name is "643842004000_Sequence_Listing.txt", the date of creation of the text file is October 4, 2012, and the size of the ASCII text file in bytes is 102,077.

FIELD OF THE INVENTION

[0003] This present invention relates to cultured recombinant cells comprising a phosphoketolase polypeptide and one or more mevalonate (MVA) pathway polypeptides capable of producing mevalonate, isoprenoid precursors, isoprene and isoprenoids and compositions that include these cultured cells, as well as methods for producing and using the same.

BACKGROUND OF THE INVENTION

[0004] *R*-Mevalonate is an intermediate of the mevalonate-dependent biosynthetic pathway that converts acetyl-CoA to isopentenyl diphosphate and dimethylallyl diphosphate. The conversion of acetyl-CoA to mevalonate can be catalyzed by the thiolase, HMG-CoA synthase and the HMG-CoA reductase activities of the upper mevalonate-dependent biosynthetic pathway (MVA pathway). Commercially, mevalonate has been used as an additive in cosmetics, for the production of biodegradable polymers, and can have value as a chiral building block for the synthesis of other chemicals. The lower mevalonate-dependent biosynthetic pathway utilizes mevalonate as substrate for generating isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are the terminal products of the mevalonate-dependent pathway. IPP and DMAPP are precursors to isoprene as well as to isoprenoids.

[0005] Isoprene (2-methyl-1,3-butadiene) is the critical starting material for a variety of synthetic polymers, most notably synthetic rubbers. Isoprene can be obtained by fractionating petroleum; however, the purification of this material is expensive and time-consuming. Petroleum cracking of the C5 stream of hydrocarbons produces only about 15% isoprene. About 800,000 tons per year of cis-polyisoprene are produced from the polymerization of isoprene; most of this polyisoprene is used in the tire and rubber industry. Isoprene is also copolymerized for use as a synthetic elastomer in other products such as footwear, mechanical products, medical products, sporting goods, and latex. Isoprene can also be naturally produced by a variety of microbial, plant, and animal species. In particular, two pathways have been identified for the natural biosynthesis of isoprene: the mevalonate (MVA) pathway and the non-mevalonate (DXP) pathway. The products of the mevalonate and non-mevalonate pathway are isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP). DMAPP can be directly converted to isoprene. IPP and DMAPP can be converted to isoprenoids.

[0006] Over 29,000 isoprenoid compounds have been identified and new isoprenoids are being discovered each year. Isoprenoids can be isolated from natural products, such as microorganisms and species of plants that use isoprenoid precursor molecules as a basic building block to form the relatively complex structures of isoprenoids. Isoprenoids are vital to most living organisms and cells, providing a means to maintain cellular membrane fluidity and electron transport. In nature, isoprenoids function in roles as diverse as natural pesticides in plants to contributing to the scents associated with cinnamon, cloves, and ginger. Moreover, the pharmaceutical and chemical communities use isoprenoids as pharmaceuticals, nutraceuticals, flavoring agents, and agricultural pest control agents. Given their importance in biological systems and usefulness in a broad range of applications, isoprenoids have been the focus of much attention by scientists.

[0007] Conventional means for obtaining mevalonate and isoprenoids include extraction from biological materials (e.g., plants, microbes, and animals) and partial or total organic synthesis in the laboratory. Such means, however, have generally proven to be unsatisfactory. In particular for isoprenoids, given the often times complex nature of their molecular structure, organic synthesis is impractical given that several steps are usually required to obtain the desired product. Additionally, these chemical synthesis steps can involve the use of toxic solvents as can extraction of isoprenoids from biological materials. Moreover, these extraction and purification

methods usually result in a relatively low yield of the desired isoprenoid, as biological materials typically contain only minute amounts of these molecules. Unfortunately, the difficulty involved in obtaining relatively large amounts of isoprenoids has limited their practical use.

[0008] Recent developments in the production of isoprene, isoprenoid precursor molecules, and isoprenoids disclose methods for the production of isoprene and isoprenoids at rates, titers, and purities that can be sufficient to meet the demands of robust commercial processes (see, for example, International Patent Application Publication No. WO 2009/076676 A2 and U.S. Patent No. 7,915,026); however, alternate pathways to improve production and yields of the same are still needed.

[0009] Provided herein are cultured recombinant cells, compositions of these cells and methods of using these cells to increase production of mevalonate as an intermediate of the mevalonate-dependent biosynthetic pathway as well as to increase production of molecules derived from mevalonate, such as isoprenoid precursors, isoprene and/or isoprenoids.

[0010] Throughout this specification, various patents, patent applications and other types of publications (e.g., journal articles) are referenced. The disclosure of all patents, patent applications, and publications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY OF THE INVENTION

[0011] The invention provided herein discloses, *inter alia*, compositions of matter comprising recombinant cells, recombinants cells and methods of making and using these recombinant cells for the production of mevalonate, isoprene, isoprenoid precursor molecules, and/or isoprenoids. Recombinant cells that have been engineered can be used to express a phosphoketolase polypeptide. The phosphoketolase enzymes of this invention can use various substrates, as described in greater detail *infra*. Accordingly, in one aspect, the invention provides for recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprene.

In one embodiment, one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In another embodiment, one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate. In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.* In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.* In another embodiment, the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide. In another embodiment, the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula.* In another embodiment, the isoprene synthase polypeptide is selected from the group consisting of *Pueraria montana* or *Pueraria lobata, Populus tremuloides, Populus alba, Populus nigra,* and *Populus trichocarpa.* In another embodiment, one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate. In another embodiment, the recombinant cells further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides. In another embodiment, the one or more nucleic acids is placed under an inducible promoter or a constitutive promoter. In another embodiment, the one or more nucleic acids is cloned into one or more multicopy plasmids. In another embodiment, the one or more nucleic acids is integrated into a chromosome of the cells. In another embodiment, the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or

yeast cells. In another embodiment, the recombinant cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae* and *Yarrowia lipolytica.*

[0012] In another aspect, the invention provides for recombinant cells capable of producing isoprenoid precursors, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprenoid precursors. In one embodiment, the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In another embodiment, the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate. In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.* In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.* In another embodiment, one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate. In another embodiment, the one or more nucleic acids is placed under an inducible promoter or a constitutive promoter. In another embodiment, one or more nucleic acids is cloned into one or more multicopy plasmids. In another embodiment, one or more nucleic acids is integrated into a chromosome of the cells. In another embodiment, the

recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells. In another embodiment, the recombinant cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae* and *Yarrowia lipolytica.*

[0013] In another aspect, the invention provides for recombinant cells capable of producing of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprenoids. In one embodiment, one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In another embodiment, one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate. In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.* In another embodiment, the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.* In another embodiment, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene. In another embodiment, the isoprenoid is a sesquiterpene. In another embodiment, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-famesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene. In another embodiment, one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-

CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate. In another embodiment, one or more nucleic acids is placed under an inducible promoter or a constitutive promoter. In another embodiment, one or more nucleic acids is cloned into one or more multicopy plasmids. In another embodiment, one or more nucleic acids is integrated into a chromosome of the cells. In another embodiment, the recombinant host cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells. In another embodiment, the recombinant host cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae* and *Yarrowia lipolytica.*

[0014] In another aspect, the invention provides for methods of producing isoprene comprising: (a) culturing any of recombinant cells listed above and described herein under conditions suitable for producing isoprene and (b) producing isoprene. In another aspect, the invention provides for methods of producing an isoprenoid precursor comprising: (a) culturing any of recombinant cells listed above and described herein under conditions suitable for producing an isoprenoid precursor and (b) producing an isoprenoid precursor. In another aspect, the invention provides for methods of producing an isoprenoid comprising: (a) culturing any of recombinant cells listed above and described herein under conditions suitable for producing an isoprenoid and (b) producing an isoprenoid.

[0015] In some aspects, the recombinant cell comprises one or more heterologous nucleic acids encoding a phosphoketolase polypeptide and one or more nucleic acids encoding one or more MVA pathway enzyme(s). In some aspects, the recombinant cells comprise a heterologous nucleic acid encoding polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate wherein the acetyl phosphate is converted to mevalonate, isoprenoid precursors, isoprene, and/or isoprenoids. In other aspects, the recombinant cells comprise a heterologous nucleic acid encoding polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate wherein the acetyl phosphate is converted to mevalonate, isoprenoid precursors, isoprene, and/or isoprenoids.

[0016] Accordingly, in certain aspects, the invention provides recombinant cells capable of enhanced production of mevalonate, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides and mevalonate. In certain embodiments, the cells produce increased amounts of mevalonate compared to mevalonate-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.*

[0017] In other aspects, the present invention provides recombinant cells capable of producing isoprenoid precursors, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides and isoprenoid precursors. In certain embodiments, the cells produce increased amounts of isoprenoid precursors compared to isoprenoid precursor-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas*

balearica, Pedobactor saltans, Streptomyces griseus, and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.*

[0018] In still other aspects, the present invention provides recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides and isoprene. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a

phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.*

[0019] In yet other aspects, the present invention provides recombinant cells capable of producing of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides wherein the cells capable of producing recoverable amounts of isoprenoids. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein the cells produce increased amounts of isoprenoids compared to isoprenoid producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the

recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*. In any of the aspects herein, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene. In one aspect, the isoprenoid is a sesquiterpene. In another aspect, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-famesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

[0020] In certain embodiments, the recombinant cells capable of producing mevalonate, isoprene, isoprenoid precursor molecules, and/or isoprenoids comprise one or more nucleic acids encoding a polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In one aspect, the one or more nucleic acids encoding a polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate is a phosphoketolase gene. In another aspect, the phosphoketolase gene is a gene from *Lactobacillus*. In another aspect, the phosphoketolase gene is from the genus *Lactobacillus reuteri*. In another aspect, the phosphoketolase gene encodes a protein having the amino acid sequence of:

MAVDYDSKKYLESVDAYWRAANYLSVGTLYLMGDPLLRQPLKAEDVKPKPIGHWGTI
VPQNFIYAHLNRVIKKYDLDMFYIEGSGHGGQVMVNNSYLDGSYTEIYPEYTQDTKGM
AKLFKHFSFPGGTASHAAPETPGSIHEGGELGYSLSHGVGAILDNPEVIAAVEIGDGEAE
TGPLMASWFSDKFINPIKDGAVLPIIQVNGFKISNPTILSWMSDEELTKYFEGMGWKPYF
VSAYKEADRDGEFKGYKPHMEVHEEMAKTLDKVVEEIKAIQKNARENNDNSLPQWPM
IIFRAPKGWTGPKTDLDGNPIENSFRAHQIPVPVSQDDMEHKDILVDWLKSYKPEELFDE
DGHPVALVEENTPEGNRRMAMNPITNGGIDPKPLVLPNYRDFAIDVQNPGSVVKQDML
EWGKYLNKMAELNPTNFRGFGPDESKSNRLYAFLDGQKRQWMESVHEPNDEDVAPQ
GRMIDSQLSEHQAEGFLEGYTLTGRHGFFATYEAFGRVVDSMLTQHMKWLRKAKDLY
WRHQYPALNFVDTSTVFQQDHNGYTHQDPGLLTHLFEKERPDLVKEYLPADTNSLMA
VSNKAFRNQECINLFVTSKHPRAQWFSIDEATQLADNGLGYIDWASTDQGTEPDVVFAS

SGTEPTEEALAAIDILHDNFPELKIRYINIIEIMRLMNTDKNPEGLTDAEFNSYFTTDKPVI
FAWHGFRDMIQALFFDRANRNVHIHSYEENGDITTPFDMRVLNELDRFHLAKDAIQSVP
GYEQKSAAFVAKMDNMINKHNHYIRSEGKDLPEVTNWTWKGLK (SEQ ID NO:2).

[0021] In other embodiments, the recombinant cells capable of producing mevalonate, isoprene, isoprenoid precursor molecules, and/or isoprenoids comprise one or more nucleic acids encoding a polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose-6-phosphate. In one aspect, the one or more nucleic acids encoding a polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate is a phosphoketolase gene. In another aspect, the phosphoketolase gene is a gene from *Bifidobacterium*. In another aspect, the phosphoketolase gene is from the genus *Bifidobacterium longum* subspecies infantis. In another aspect, the phosphoketolase gene encodes a protein having the amino acid sequence of:

MTSPVIGTPWKKLSAPVSEEALEGVDKYWRVANYLSIGQIYLRSNPLMKEPFTREDVKH
RLVGHWGTTPGLNFLIGHINRFIADHGQNTVIIMGPGHGGPAGTSQSYLDGTYTETFPKI
TKDEAGLQKFFRQFSYPGGIPSHFAPETPGSIHEGGELGYALSHAYGAIMDNPSLFVPAIV
GDGEAETGPLATGWQSNKLVNPRTDGIVLPILHLNGYKIANPTILSRISDEELHEFFHGM
GYEPYEFVAGFDDEDHMSIHRRFAELWETIWDEICDIKATAQTDNVHRPFYPMLIFRTP
KGWTCPKYIDGKKTEGSWRSHQVPLASARDTEAHFEVLKNWLESYKPEELFDANGAV
KDDVLAFMPKGELRIGANPNANGGVIRDDLKLPNLEDYEVKEVAEFGHGWGQLEATR
SLGAYTRDIIKNNPRDFRIFGPDETASNRLQASYEVTNKQWDAGYISDEVDEHMRVSGQ
VVEQLSEHQMEGFLEAYLLTGRHGIWSSYESFVHVIDSMLNQHAKWLEATVREIPWRK
PIASMNLLVSSHVWRQDHNGFSHQDPGVTSVLLNKCFHNDHVIGIYFATDANMLLAIAE
KCYKSTNKINAIIAGKQPAAT
WLTLDEARAELEKGAAAWDWASTAKTNDEAEIVLAAAGDVPTQEIMAASDKLKELGI
KFKVVNVVDLLSLQSAKENDEALSNEEFADIFTADKPVLFAYHSYAHDVRGLIYDRPNH
DNFNVHGYEEEGSTTTPYDMVRVNRIDRYELTAEALRMIDADKYADKIDELEKFRDEA
FQFAVDKGYDHPDYTDWVYSGVNTGKKGAVTATAATAGDNE (SEQ ID NO:4).

[0022] In one aspect, the invention described herein provides for recombinant cells capable of producing mevalonate, isoprenoids precursor, isoprene and/or isoprenoids comprising one or more heterologous nucleic acids encoding a polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate and one or more

nucleic acids encoding: (a) a peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA; (b) one or more mevalonate (MVA) pathway polypeptides; wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides and synthesis of mevalonate, isoprenoids precursor, isoprene and/or isoprenoids. In one aspect, the one or more nucleic acids encoding a polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate is a phosphoketolase gene. In one aspect, the peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA is a peptide having derived from *Streptomyces* sp. CL190. In one aspect, the peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA is a peptide having the amino acid sequence of SEQ ID NO:5.

[0023] In another aspect, the invention provides for recombinant cells capable of producing mevalonate, isoprenoids precursor, isoprene and/or isoprenoids isoprene comprising one or more heterologous nucleic acids encoding a polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate and one or more nucleic acids encoding: (a) a peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA; (b) one or more mevalonate (MVA) pathway polypeptides; wherein culturing of said recombinant cells in a suitable media provides for the production of said polypeptides and synthesis of mevalonate, isoprenoids precursor, isoprene and/or isoprenoids. In one aspect, the one or more nucleic acids encoding a polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate is a phosphoketolase gene. In one aspect, the peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA is a peptide having derived from *Streptomyces* sp. CL190. In one aspect, the peptide that synthesizes acetoacetyl-CoA from malonyl-CoA and acetyl-CoA is a peptide having the acid sequence of SEQ ID NO:5.

[0024] In another aspect, the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity encodes a polypeptide having an amino acid sequence with 80% or more identity to the amino acid sequence of SEQ ID NO:2 and having a function of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In certain embodiments, the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity encodes a polypeptide having 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:2. In another aspect, the one or more heterologous nucleic

acids encoding a polypeptide having phosphoketolase activity encodes a polypeptide having an amino acid sequence with 80% or more identity to the amino acid sequence of SEQ ID NO: 4 and having a function of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate. In certain embodiments, the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity encodes a polypeptide having 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:4.

[0025] In any of the aspects herein, the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide. In any of the aspects herein, the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula*. In any of the aspects herein, the isoprene synthase polypeptide is selected from the group consisting of *Pueraria montana* or *Pueraria lobata, Populus tremuloides, Populus alba, Populus nigra*, and *Populus trichocarpa*. In another aspect, the plant isoprene synthase polypeptide is a kudzu isoprene synthase polypeptide. In another aspect, the isoprene synthase is an engineered isoprene synthase, such as those described in U.S. Pat. Publ. No. 2010/0003716 and U.S. Pat. Publ. No. 2011/0076743.

[0026] In any of the aspects herein, the invention provides a recombinant host cell, or progeny thereof, comprising cells engineered for increased carbon flux towards mevalonate production wherein the activity of one or more enzymes from the group consisting of: (a) citrate synthase, (b) phosphotransacetylase; (c) acetate kinase; (d) lactate dehydrogenase; (e) glyceraldehyde 3-phosphate dehydrogenase, (f) pyruvate dehydrogenase, (g) Phosphogluconate dehydratase, (h) 2-keto-3-deoxygluconate 6-phosphate aldolase, (i) phsophofructokinase, (j) transketolase, (k) transaldolase, (l) ribulose-5-phosphate-epimerase, and/or (m) ribose-5-phosphate epimerase is modulated.

[0027] In any of aspects herein, the cells can further comprise an *mvaE* gene and an *mvaS* gene selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

[0028] In certain aspects, the one or more nucleic acids encoding one or more MVA pathway polypeptides is a heterologous nucleic acid. In other aspects, the one or more nucleic acids encoding one or more MVA pathway polypeptides is a copy of an endogenous nucleic acid. In any of the aspects herein, one or more MVA pathway polypeptides can be selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate; and (g) an enzyme that converts isopentenyl pyrophosphate to dimethylallyl diphosphate. In any of the aspects herein, one or more MVA pathway polypeptides is selected from (a) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (b) an enzyme that converts HMG-CoA to mevalonate; (c) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (d) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (e) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

[0029] In any of the aspects herein, the enzyme that phosphorylates mevalonate to mevalonate 5-phosphate can be selected from the group consisting of *M. mazei* mevalonate kinase, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, *yeast* mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, and *Streptomyces* mevalonate kinase polypeptide, *Streptomyces CL190* mevalonate kinase polypeptide, or *M. burtonii* mevalonate kinase. In any of the aspects herein, the enzyme that phosphorylates mevalonate to mevalonate 5-phosphate is *M. mazei* mevalonate kinase.

[0030] In any of the aspects herein, the recombinant cells can further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides. In one aspect, one or more nucleic acids that encode for one or more DXP pathway polypeptides is a heterologous nucleic acid. In another aspect, the one or more nucleic acids encoding one or more DXP pathway polypeptides is a copy of an endogenous nucleic acid. In another aspect, the one or more DXP pathway polypeptides is selected from (a) 1-deoxy-D-

xylulose-5-phosphate synthase (DXS), (b) 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), (c) 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (MCT), (d) 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK), (e) 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MCS), (f) 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase (HDS), and (g) 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR). In another aspect, the DXP pathway polypeptide is DXS.

[0031] In any of the aspects herein, the one or more heterologous nucleic acids is placed under an inducible promoter or a constitutive promoter. In any of the aspects herein, the one or more heterologous nucleic acids is cloned into one or more multicopy plasmids. In any of the aspects herein, the one or more heterologous nucleic acids is integrated into a chromosome of the cells.

[0032] In any of the aspects herein, the recombinant host cell is a bacterial, algal, fungal, yeast, or cyanobacterial cell. In one aspect, the host cell is a bacterial cell. In another aspect, the bacterial cell is a gram-positive bacterial cell or gram-negative bacterial cell. In another aspect, the bacterial cell is selected from the group consisting of *E. coli*, *L. acidophilus*, *P. citrea*, *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, B. *clausii*, *B. halodurans*, *B. megaterium*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. thuringiensis*, *S. albus*, *S. lividans*, *S. coelicolor*, *S. griseus*, *Pseudomonas sp.*, and *P. alcaligenes* cells. In another aspect, the bacterial cell is an *E. coli* cell. In another aspect, the bacterial cell is an *L. acidophilus* cell. In another aspect, the is an algal cell. In another aspect, the algal cell is selected from the group consisting of green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, or dinoflagellates. In another aspect, the host cell is a fungal cell. In another aspect, the fungal cell is a filamentous fungi. In another aspect, the host cell is a yeast cell. In another aspect, the yeast cell is selected from the group consisting of *Saccharomyces sp., Schizosaccharomyces sp., Pichia sp., or Candida* sp. In another aspect, the yeast cell is a *Saccharomyces cerevisiae* cell.

[0033] In another aspect, the invention provides for a recombinant host cell capable of producing an isoprenoid comprising one or more nucleic acids encoding a polypeptide capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate and one or more nucleic acids encoding: (a) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase; and (b) one or more nucleic acids encoding one or more mevalonate

(MVA) pathway polypeptides, wherein culturing of said recombinant host cell in a suitable media provides for production of said polypeptides and synthesis of one or more isoprenoid(s). In one aspect, the one or more nucleic acids encoding one or more MVA pathway polypeptides of (b) is a heterologous nucleic acid. In any of the aspects herein, the one or more MVA pathway polypeptides is selected from the group consisting of (a) an enzyme that converts acetoacetyl-CoA to HMG-Co-A; (b) an enzyme that converts HMG-CoA to mevalonate; (c) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (d) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (e) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

[0034] In another aspect, the invention provides for a recombinant host cell capable of producing an isoprenoid comprising one or more nucleic acids encoding a polypeptide capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate and one or more nucleic acids encoding: (a) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase; and (b) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides, wherein culturing of said recombinant host cell in a suitable media provides for production of said polypeptides and synthesis of one or more isoprenoid(s). In one aspect, the one or more nucleic acids encoding one or more MVA pathway polypeptides of (b) is a heterologous nucleic acid. In any of the aspects herein, the one or more MVA pathway polypeptides is selected from the group consisting of (a) an enzyme that converts acetoacetyl-CoA to form HMG-Co-A; (b) an enzyme that converts HMG-CoA to mevalonate; (c) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (d) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (e) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

[0035] In another aspect, the invention provides for methods of producing mevalonate comprising: (a) culturing a recombinant host cell comprising one or more heterologous nucleic acids encoding (i) a polypeptide having phosphoketolase activity; (ii) and (b) producing mevalonate. In one aspect, the method further comprises recovering the mevalonate produced by the recombinant host cell. In certain embodiments, the methods provide for production of increased amounts of mevalonate compared to mevalonate-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding

phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.*

[0036] In other aspects, the invention provides for methods of producing isoprenoid precursors comprising: (a) culturing a recombinant host cell comprising (i) one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity; (ii) and one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (b) producing an isoprenoid precursor. In one aspect, the method further comprises recovering the isoprenoid produced by the recombinant host cell. In certain embodiments, the methods comprise recombinant cells that produce increased amounts of isoprenoid precursors compared to isoprenoid precursor-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more

copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*.

[0037] In still other aspects, the present invention provides for methods of producing isoprene comprising: (a) culturing a recombinant host cell comprising: (i) one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity; (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway; and (iii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, and (b) producing isoprene. In one aspect, the method further comprises recovering the isoprenoid produced by the recombinant host cell. In certain embodiments, the methods comprise recombinant cells capable of enhanced production of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise

one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.*

[0038] In yet other aspects, the present invention provides methods for producing of isoprenoids comprising: (a) culturing a recombinant host cell comprising: (i) one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity; (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway ; and (iii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, and (b) producing an isoprenoid. In one aspect, the method further comprises recovering the isoprenoid produced by the recombinant host cell. In certain embodiments, the methods comprise recombinant cells capable of enhanced production of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein the cells produce increased amounts of isoprenoids compared to isoprenoid producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In any of the aspects herein, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene. In one aspect, the isoprenoid is a sesquiterpene. In

another aspect, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-famesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

[0039] In another aspect, the invention provides for methods of producing mevalonate using any of of the recombinant cells described herein. In another aspect, the invention provides for methods of producing isoprenoid precursors using any of of the recombinant cells described herein. In another aspect, the invention provides for methods of producing isoprene using any of of the recombinant cells described herein. In another aspect, the invention provides for methods of producing isoprenoid precursors using any of of the recombinant cells described herein. In another aspect, the invention provides for methods of producing isoprenoids using any of of the recombinant cells described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] Figure 1 depicts the metabolic pathway for glucose metabolism in *E. coli*. Reactions associated with ATP or NADH production or use as well as key enzymes involved in carbon flux are represented. Abbreviation 'P5P' indicate the pool of metabolites consisting of xylulose 5 phosphate, ribulose-5 phosphate, and ribose-5 phosphate. Abbreviation 'Triose-3P' indicates the pool of metabolites consisting of glyceraldehyde 3 phosphate and dihydroxyacetone phosphate.

[0041] Figure 2 depicts an engineered metabolic pathway with phosphoketolase (*PKT*) present. PKTs have been classified into two types based on substrate preference: xylulose-5-phosphate (X5P) phosphoketolases (EC 4.1.2.9), which only act on X5P, and xylulose-5-phosphate /fructose-6-phosphate (F6P) phosphoketolases (EC 4.1.2.22), which act on both X5P and F6P with comparable activities. Acetyl phosphate (Ac-P) formed from F6P and/or X5P in *PKT*-catalyzed reaction(s) is subsequently converted to acetyl-CoA for use in the MVA pathway. Other products of *PKT*-catalyzed reaction, namely glyceraldehyde 3-phosphate (GAP) and erythrose 4-phosphate (E4P) produced from X5P and F6P, respectively, can be recycled through manipulated metabolic pathways to maximize yield.

[0042] Figure 3 depicts the reactions involving phosphoketolase reactants and products. Reactions (1) and (2) depict reactions catalyzed by phosphoketolase enzymes. Reaction (3) depicts the conversion of acetyl-P to acetyl-Coa, which is catalyzed by the Phosphotransacetylase (*pta*) enzyme.

[0043] Figure 4 is a table containing a representative list of phosphoketolase polypeptides. This table is merely representative and not intended to be limiting as any phosphoketolase polypeptide that converts xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate and/or converts fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate is contemplated for use in the present invention.

[0044] Figure 5 depicts an engineered pathway in which erythrose 4 phosphate (E4P) and glucose-3 phosphate (G3P) generated in phosphoketolase-catalyzed reaction (PKT) are converted back to PKT substrates by sedoheptulose-1,7-bisphosphatase/fructose-1,6 – bisphosphate aldolase (SFA) and sedoheptulose-1,7-bisphosphatase/fructose-1,6 –bisphosphate phosphatase (SFP). Other abbreviations used in this figure indicate xylulose 5-phosphate (X5P), sedoheptulose-1,7-bisphosphate (S1,7BP), sedoheptulose-7-phosphate (S7P), fructose-1,6 – bisphosphate (F1,6BP), fructose-6 –phosphate (F6P), dihydroxyacetone phosphate (DHAP). X indicates the attenuated or deleted enzymatic reactions.

[0045] Figure 6 depicts the plasmid map of pCMP1090, expressing *Bifidobacterium infantis* phosphoketolase.

[0046] Figure 7 depicts the plasmid map of pCMP1029, expressing *Lactobacillus reuteri* phosphoketolase.

[0047] Figure 8 depict the results of intracellular acetyl-phosphate (mM) in expressing phosphoketolase from *B. infantis* or *L. reuteri*, and the control strain.

[0048] Figure 9 depicts the Growth curve (measured as OD as a function of time) of the strains expressing phosphoketolase from *B. infantis* or *L. reuteri*, and the control strain.

[0049] Figure 10 depicts mevalonate concentration (g/L) in the shake flasks of strains expressing phosphoketolase from *B. infantis* or *L. reuteri*, and the control strain.

[0050] Figure 11 depicts the yield of mevalonate on glucose achieved by the phosphoketolase expressing strain (closed black squares) compared the control strain (open diamonds) in the 15-L fermentation over time. Strains were run under the same conditions. Overall yield was calculated using the following formula: %wt Yield on glucose = Mevalonate total(t)/[(Feed Wt(0)-Feed Wt(t)+83)*0.59)], where 0.59 is the wt% of glucose in the glucose feed solution and 83 is the grams of this feed batched into the fermentor at t=0.

[0051] Figure 12 depicts mevalonate titer achieved by the phosphoketolase expressing strain (closed black squares) compared the control strain (open diamonds) in the 15-L fermentation over time. Strains were run under the same conditions. Titer was calculated using the following formula: **Titer** = grams Mevalonate / Liter of whole fermentor broth.

[0052] Figure 13 depicts Cell Productivity Index (CPI) achieved by the phosphoketolase expressing strain (closed black squares) compared the control strain (open diamonds) in the 15-L fermentation over time. Strains were run under the same conditions. Cell Productivity Index (CPI) was calculated using the following formula: CPI = total grams Mevalonate / total grams dry cell weight.

[0053] Figure 14 provides a graph showing that accumulated acetate in the fermentation broth was substantially lower when using the phosphoketolase expressing strain (closed black squares) as compared the control strain (open diamonds) in the 15-L fermentation over time. Strains were run under the same conditions. Acetate was measured by HPLC and the concentration was reported using the following formula: [Acetate] = grams acetate / Liter of whole fermentor broth.

[0054] Figure 15 provides the nucleotide sequence for the nucleic acid sequence encoding a phosphoketolase from *Lactobacillus reuteri* (SEQ ID NO:1).

[0055] Figure 16 provides the amino acid sequence for a phosphoketoase enzyme from *Lactobacillus reuteri* (SEQ ID NO:2).

[0056] Figure 17 provides the nucleotide sequence for the nucleic acid sequence encoding a phosphoketoase from *Bifidobacterium longum* (SEQ ID NO:3).

[0057] Figure 18 provides the amino acid sequence for a phosphoketoase enzyme from *Bifidobacterium longum* (SEQ ID NO:4).

[0058] Figure 19 provides the amino acid sequence for an aceto-acetyl-CoA synthase enzyme from *Streptomyces sp.* CL190 (SEQ ID NO:5).

[0059] Figure 20 provides the nucleotide sequence for the nucleic acid sequence encoding mvaE from *L. grayi* (SEQ ID NO:6).

[0060] Figure 21 provides the nucleotide sequence for the nucleic acid sequence encoding mvaE from *E. faecium* (SEQ ID NO:7).

[0061] Figure 22 provides the nucleotide sequence for the nucleic acid sequence encoding mvaE from *E. gallinarum* (SEQ ID NO:8).

[0062] Figure 23 provides the nucleotide sequence for the nucleic acid sequence encoding mvaE from *E. casseliflavus* (SEQ ID NO:9).

[0063] Figure 24 provides the nucleotide sequence for the nucleic acid sequence encoding mvaS from *L. grayi* (SEQ ID NO:10).

[0064] Figure 25 provides the nucleotide sequence for the nucleic acid sequence encoding mvaS from *E. faecium* (SEQ ID NO:11).

[0065] Figure 26 provides the nucleotide sequence for the nucleic acid sequence encoding mvaS from *E. gallinarum* (SEQ ID NO:12).

[0066] Figure 27 provides the nucleotide sequence for the nucleic acid sequence encoding mvaS from *E. casseliflavus* (SEQ ID NO:13).

[0067] Figure 28 provides the nucleotide sequence for pCMP1090 (SEQ ID NO:15).

[0068] Figure 29 provides the nucleotide sequence for pCMP1029 (SEQ ID NO:16).

[0069] Figure 30 provides the nucleic acid sequence encoding a phosphoketolase from *E. gallinarum* (SEQ ID NO:17).

[0070] Figure 31 provides the nucleic acid sequence encoding a phosphoketolase from *N. punctiforme* (SEQ ID NO:18).

[0071] Figure 32 provides the nucleic acid sequence encoding a phosphoketolase from *R. palustris* (SEQ ID NO:19).

[0072] Figure 33 provides the nucleic acid sequence encoding a phosphoketolase from *Pantoea* (SEQ ID NO:20).

[0073] Figure 34 provides the nucleic acid sequence encoding a phosphoketolase from *M. paludis* (SEQ ID NO:21).

[0074] Figure 35 provides the nucleic acid sequence encoding a phosphoketolase from *T. fusca* (SEQ ID NO:22).

[0075] Figure 36 provides the nucleic acid sequence encoding a phosphoketolase from *B. breve* (SEQ ID NO:23).

[0076] Figure 37 provides the nucleic acid sequence encoding a phosphoketolase from *R. aquatilis* (SEQ ID NO:24).

[0077] Figure 38 provides the nucleic acid sequence encoding a phosphoketolase from *B. animalis* (SEQ ID NO:25).

[0078] Figure 39 provides the nucleic acid sequence encoding a phosphoketolase from *G. vaginalis* (SEQ ID NO:26).

[0079] Figure 40 provides the nucleic acid sequence encoding a phosphoketolase from *S. avermitilis* (SEQ ID NO:27).

[0080] Figure 41 provides the nucleic acid sequence encoding a phosphoketolase from *C. acetobutylicum* (SEQ ID NO:28).

[0081] Figure 42 provides the nucleic acid sequence encoding a phosphoketolase from *L. paraplantarum* (SEQ ID NO:29).

[0082] Figure 43 is a graph showing the activity of PKLs from *B. longum* and *E. gallinarum* in the presence of F6P substrate.

[0083] Figure 44 is a graph showing the activity of PKLs from *E. gallinarum* in the presence of F6P and X5P substrates.

[0084] Figure 45 is a graph showing metabolite formation by a strain expressing *B. longum* PKL in the presence of S7P substrate.

[0085] Figure 46 is a panel of graphs showing MVA yield by strains expressing *B. longum* PKL (EWL1319), *E. gallinarum* PKL (EWL1341), *N. punctiforme* PKL (EWL1344), *R. palustris* PKL (EWL1347), *Pantoea* PKL (EWL1350), or *T. fusca* PKL (EWL1353) as compared to a control strain not expressing PKL (CHL875).

[0086] Figure 47 is a series of SDS-PAGE coomasie stained gels showing protein expression in strains expressing phosphoketolase. A) control strain CHL875 not expressing PKL and strain EWL1319 expressing *B. longum* PKL, B) strain EWL1341 expressing *E. gallinarum* PKL and strain EWL1344 expressing *N. punctiforme* PKL, C) strain EWL1347 expressing *R. palustris* PKL and strain EWL1350 expressing *Pantoea* PKL, and D) strain EWL1353 expressing *T. fusca* PKL with increasing IPTG induction.

[0087] Figure 48 is a graph showing protein expression and MVA yield by a strain expressing *B. longum* PKL. A) MVA yield by an MVA producing strain expressing *B. longum* PKL (closed square) as compared to an MVA producing control strain not expressing PKL (closed diamonds) with increasing IPTG induction. B) Protein expression of *B. longum* PKL in the soluble fraction of whole cell lysates from strain EWL1319. C) Protein expression of *B. longum* PKL in the insoluble fraction of whole cell lysates from strain EWL1319.

[0088] Figure 49 contains an SDS-PAGE coomasie stained gel and graph showing protein expression and MVA yield by a strain expressing *E. gallinarum* PKL. A) MVA yield by an MVA producing strain expressing *E. gallinarum* PKL (closed square) as compared to an MVA producing control strain not expressing PKL (closed diamonds) with increasing IPTG induction. B) Protein expression in whole cell lysates from strain EWL1341 expressing *E. gallinarum*.

[0089] Figure 50 contains an SDS-PAGE coomasie stained gel and graph showing protein expression and MVA yield by a strain expressing *C. acetobutylicum* PKL. A) MVA yield by an MVA producing strain expressing *C. acetobutylicum* PKL (closed square) as compared to an MVA producing control strain not expressing PKL (closed diamonds) with increasing IPTG induction. B) Protein expression in whole cell lysates from strain EWL1341 expressing *C. acetobutylicum*.

[0090] Figure 51 is a graph showing cumulative yield of MVA on glucose achieved by a strain expressing *C. acetobutylicum* PKL in each15-L fermentation over time. Closed circle indicates run 20121058: EWL1359 induced at 400μM IPTG; x symbol indicates run 20121057: EWL1359 induced at 100μM IPTG; open triangle indicates run 20121056: EWL1359 with no added IPTG; and open diamond indicates run 20121059: CHL875 induced at 100μM IPTG.

[0091] Figure 52 is a graph showing cumulative yield of MVA on glucose achieved by a strain expressing *E. gallinarum* PKL in each15-L fermentation over time. Closed square indicates 20120979: EWL1341 induced at 100μM IPTG; x symbol indicates run 20120978: EWL1341 induced at 50μM IPTG; open square indicates run 20120977: EWL1341 with no added IPTG; open diamond indicates run 20120976: CHL875 with no added IPTG; and closed diamond indicates run 20120821: CHL875 with no added IPTG.

[0092] Figure 53 is a graph showing cumulative yield of MVA on glucose achieved by a strain expressing *E. gallinarum* PKL or *C. acetobutylicum* PKL plotted against the amount of IPTG added. Closed triangles indicate strain EWL1359. Closed squares indicate strain EWL1341. Open diamonds indicate strain CHL875.

[0093] Figure 54 is a graph showing cumulative yield of MVA on glucose achieved by a strain expressing *E. gallinarum* PKL or *C. acetobutylicum* PKL plotted against the phosphoketolase activity. Closed triangles indicate strain EWL1359. Closed squares indicate strain EWL1341. Open diamonds indicate strain CHL875.

[0094] Figure 55 is a graph showing cell performance index achieved by a strain expressing *C. acetobutylicum* PKL in each15-L fermentation over time. Closed circle indicates run 20121058: EWL1359 induced at 400μM IPTG; x symbol indicates run 20121057:

EWL1359 induced at 100μM IPTG; open triangle indicates run 20121056: EWL1359 with no added IPTG; and open diamond indicates run 20121059: CHL875 induced at 100μM IPTG.

[0095] Figure 56 is a graph showing cell performance index achieved by a strain expressing *E. gallinarum* PKL in each15-L fermentation over time. Closed square indicates 20120979: EWL1341 induced at 100μM IPTG; x symbol indicates run 20120978: EWL1341 induced at 50μM IPTG; open square indicates run 20120977: EWL1341 with no added IPTG; open diamond indicates run 20120976: CHL875 with no added IPTG; and closed diamond indicates run 20120821: CHL875 with no added IPTG.

[0096] Figure 57 depicts the plasmid map of pEWL1418, expressing *B. longum* phosphoketolase and *P. alba* isoprene synthase variant.

[0097] Figure 58 depicts the plasmid map of pEWL1421, expressing *E. gallinarum* phosphoketolase and *P. alba* isoprene synthase variant.

[0098] Figure 59 depicts the plasmid map of pEWL1438, expressing *E. gallinarum* phosphoketolase, *P. alba* isoprene synthase variant, and *M. mazei* mevanolate kinase.

[0099] Figure 60 depicts the plasmid map of pEWL1436, expressing *C. acetobutylicum* phosphoketolase and *P. alba* isoprene synthase variant.

[0100] Figure 61 depicts the plasmid map of pEWL1440, expressing *C. acetobutylicum* phosphoketolase, *P. alba* isoprene synthase variant, and *M. mazei* mevanolate kinase.

[0101] Figure 62 is is a panel of graphs showing isoprene yield by strains expressing phosphoketolase. A) Isoprene yield by strains expressing *B. longum* PKL (strain EWL1427) or *E. gallinarum* PKL (strain EWL1430). B) Isoprene yield by strains expressing *C. acetobutylicum* PKL (EWL1446). Control strain MCM2158 does not express PKL.

[0102] Figure 63 is a series of SDS-PAGE coomasie stained gels showing protein expression as induced by IPTG. A) strains expressing *B. longum* PKL (EWL1427) or *E. gallinarum* PKL (EWL1430). B) strains expressing *C. acetobutylicum* PKL (EWL1446). Control strain MCM2158 does not express PKL.

[0103] Figure 64 contains an SDS-PAGE coomasie stained gel and graph showing protein expression and isoprene yield by strains expressing a PKL. A) isoprene yield by an isoprene producing strain expressing *E. gallinarum* PKL (strain EWL1449) or *C. acetobutylicum* PKL (EWL1452) as compared to an MVA producing control strain not expressing PKL (strain DW719) with increasing IPTG induction. B) Protein expression in whole cell lysates from strain EWL1452 and EWL1449.

[0104] Figure 65 is a graph showing cumulative yield of isoprene on glucose achieved by strains expressing *B. longum* PKL (strain EWL1427) or *E. gallinarum* PKL (strain EWL1430) in each15-L fermentation over time. Closed triangle indicates run 20121136: EWL1430 induced at 100μM IPTG; closed square indicates run 20121135: EWL1427 induced at 100μM IPTG; and open diamond indicates 20121134: MCM2158 induced at 100μM IPTG.

[0105] Figure 66 is a graph showing instantantaneous yield of isoprene on glucose achieved by strains expressing *B. longum* PKL (strain EWL1427) or *E. gallinarum* PKL (strain EWL1430) in each15-L fermentation over time. Open triangle indicates run 20121136: EWL1430 induced at 100μM IPTG; open square indicates run 20121135: EWL1427 induced at 100μM IPTG; and open diamond indicates 20121134: MCM2158 induced at 100μM IPTG.

[0106] Figure 67 is a graph showing cell performance index achieved achieved by a strain expressing *B. longum* PKL (strain EWL1427) or *E. gallinarum* PKL (strain EWL1430) in each15-L fermentation over time. Closed triangle indicates run 20121136: EWL1430 induced at 100μM IPTG; closed square indicates run 20121135: EWL1427 induced at 100μM IPTG; and open diamond indicates run 20121134: MCM2158 induced at 100μM IPTG. gDCW indicates total grams dry cell weight.

[0107] Figure 68 is a series of graphs showing *E. gallinarum* PKL in vitro activity. A) In vitro AcP specific productivity. B) In vitro PKL specific activity.

[0108] Figure 69 is a series of graphs showing *C. acetobutylicum* PKL in vitro activity. A) In vitro AcP specific productivity. B) In vitro PKL specific activity.

[0109] Figure 70 is a graph showing in vitro AcP specific productivity in isoprene producing strains expressing *B. longum* PKL or *E. gallinarum* PKL.

[0110] Figure 71 is a graph showing in vitro PKL activity in isoprene producing strains expressing *B. longum* PKL or *E. gallinarum* PKL.

[0111] Figure 72 is a diagram depicting host mutations that are preferably upregulated to increase carbon flux through the phosphoketolase pathway. Genes of interest for modulating carbon flux include moduribose-5-phosphate isomerase A (rpiA), D-ribulose-5-phosphate 3-epimerase (rpe), transketolase A (tktA), transaldolase B (tal B), and/or phosphate acetyltransferase (pta).

[0112] Figure 73 is a diagram depicting host mutations that are preferably downregulated to increase carbon flux through the phosphoketolase pathway. Genes of interest for modulating carbon flux include glucose-6-phosphate dehydrogenase (zwf), 6-phosphofructokinase-1 (pfkA), fructose bisphosphate aldolase (fba), glyceraldehyde-3-phosphate dehydrogenase A (gapA), Acetate kinase (ackA), citrate synthase (gltA) and/or the pts operon.

DETAILED DESCRIPTION

[0113] The invention provided herein discloses, *inter alia,* compositions and methods for the production of mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids in recombinant cells that have been engineered to express a phosphoketolase polypeptide. The phosphoketolase enzymes of this invention can use various substrates, as described in greater detail *infra.* In certain embodiments, the invention provides for compositions and methods for the production of mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids in recombinant cells that have been engineered to express a phosphoketolase polypeptide capable of catalyzing the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate. In other embodiments, the invention provides for compositions and methods for the production of mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids in recombinant cells that have been engineered to express a phosphoketolase polypeptide capable of catalyzing the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. In still other embodiments, the invention provides for compositions and methods for the production of mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids in recombinant cells that have been engineered to express a phosphoketolase polypeptide capable of catalyzing the conversion of sedoheptulose-7-phosphate to ribose-5-phosphate and acetyl

phosphate. In still other embodiments, the invention provides for compositions and methods for the production of mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids in recombinant cells that have been engineered to express a phosphoketolase polypeptide capable of catalyzing the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate and/or the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate and/or the conversion of sedoheptulose-7-phosphate to ribose-5-phosphate and acetyl phosphate.

[0114] Recombinantly expressed phosphoketolase has been used to engineer metabolic pathways in host cells. See U.S. 7,785,858. Sonderegger et al. (*Applied and Environmental Microbiology*, 2004, 70:5, 2892-97) describe the use of phosphoketolase in *Saccharomyces* cerevisiae for the overproduction of ethanol. Fleige et al. (*Appl Microbial Biotechnol.*, 2011, 91:3, 769-76) describe the expression of a bifidobacterium phosphoketolase gene (Meile et al., *supra*) in a modified *Ralstonia eutropha* strain which restored the capability for the organism to utilize fructose as a sole carbon source for growth. However, utilization of a phosphoketolase to increase carbon flux into the mevalonate pathway has not been described.

[0115] The mevalonate-dependent biosynthetic pathway is particularly important for the production of the mevalonate and other isoprenoid precursor molecules, *e.g.*, dimethylallyl diphosphate (DMAPP) and isopentenyl pyrophosphate (IPP). The enzymes of the upper mevalonate pathway convert acetyl CoA, produced from glucose, into mevalonate via three enzymatic reactions. Without being bound to theory, it is believed that increased biosynthesis of acetyl CoA by the use of a phosphoketolase polypeptide can result in increased productivity of the upper mevalonate-dependent biosynthetic pathway which will substantially increase biosynthesis of mevalonate and, consequently, of downstream isoprenoid precursor molecules such as DMAPP and IPP. The increased yield of mevalonate production by this alternate pathway is therefore advantageous for commercial applications.

[0116] Theoretically, three molecules of acetyl-CoA can be derived from a single molecule of glucose in a balanced reaction. However, organisms typically produce only up to two molecules of acetyl-CoA, with the remainder mass being lost as CO_2. The release of CO_2 occurs during the formation of acetyl-CoA from pyruvate, a reaction catalyzed by pyruvate dehydrogenase. The loss of one carbon atom results in decreased production yields of mevalonate, isoprenoid precursors, isoprene, and isoprenoid molecules. An exception to this

reaction loss is the Wood-Ljungdahl pathway, which relies on carbon monoxide dehydrogenase and acetyl-CoA synthase enzymes to reduce the carbon dioxide to acetyl-CoA in anaerobic acetogens.

[0117] The present invention provides an alternate metabolic process which can potentially produce three molecules of acetyl-CoA from one molecule of glucose using a pathway which does not rely on the Wood-Ljungdahl pathway enzymes. Instead, it makes use of a phosphoketolase enzyme found in certain organisms, particularly among Bifidobacteria [see, for example, Biology of the Prokaryotes (ed. Lengeler, Drews and Schlegel); Blackwell Science, New York ,1999, p. 299-301; Meile et al., *J. of Bacteriology*, 2001, 183:9, 2929-36; Jeong et al., *J. Microbiol. Biotechnol.*, 2007, 17:5, 822-829]. Phosphoketolase enzymes allow for formation of acetyl-CoA (via acetyl-phosphate) from xylulose 5-phosphate or fructose 6-phosphate rather than through oxidation of pyruvate as in typical metabolism.

[0118] Phosphoketolases have been classified into two types based on their substrate preference: xylulose-5-phosphate (X5P) phosphoketolases, which only act on X5P, and X5P/fructose-6-phosphate (F6P) phosphoketolases, which can act on both X5P and F6P (Suzuki et al., *Acta Cryst. F66*, 2010, 66:8, 941-43). Phosphoketolases catalyze the cleavage of X5P or F6P utilizing inorganic phosphate (P_i) to produce acetyl phosphate (acetyl-P), H_2O and glyceraldehyde 3-phosphate or erythrose 4-phosphate. The high-energy metabolite acetyl-P is subsequently converted to acetic acid by acetate kinase to produce ATP from ADP in the pathway. In addition to acetyl-phosphate, the glyceraldehyde 3-phosphate produced from the enzymatic reaction can be recycled through manipulated metabolic pathways so that the maximum yield of 3 acetyl-CoA per glucose can be achieved. Significantly, acetyl-CoA production by phosphoketolase eliminates the loss of carbon (e.g. CO_2) as observed from pyruvate dehydrogenase mediated reactions.

[0119] As further detailed herein, phosphoketolases can also act upon sedoheptulose-7-phosphate to convert it to ribose-5-phosphate and acetyl phosphate. A non-limiting example of such a phosphoketolase is Bifidobacterium longum phosphoketolase, which has catalytic activity with sedoheptulose-7-phosphate.

[0120] The present invention is directed to the use of phosphoketolase enzymes in the production of mevalonate, isoprenoid precursors, isoprene and/or isoprenoids to enhance product

yield. In particular, the theoretical isoprene product yield is enhanced as represented by the following balanced equations (with the assumption that an organism is capable of producing ATP from the complete oxidation of 1 mol glucose to 6 mol CO_2):

MVA pathway only

1.5 Glucose + 2.00 O_2 → 1.00 Isoprene + 4.00 CO_2 + 5.00 H_2O

Theoretical yield – 0.252 g Isoprene/g Glucose

DXP pathway

1.25 Glucose + 0.50 O_2 → 1.00 Isoprene + 2.50 CO_2 + 3.50 H_2O

Theoretical yield – 0.302 g Isoprene/g Glucose

MVA + Phosphoketolase pathways

1.22 Glucose + 0.33 O_2 → 1.00 Isoprene + 2.33 CO_2 + 3.32 H_2O

Theoretical yield – 0.309 g Isoprene/g Glucose

[0121] Accordingly, in certain aspects, the invention provides recombinant cells capable of enhanced production of mevalonate, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein the cells produce increased amounts of mevalonate compared to cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0122] In other aspects, the present invention provides recombinant cells capable of enhanced production of isoprenoid precursors, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein the cells produce increased amounts of isoprenoid precursors compared to cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0123] In still other aspects, the present invention provides recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids

encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells are capable of producing recoverable amounts of isoprene. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0124] In yet other aspects, the present invention provides recombinant cells capable of producing of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein the cells are capable of producing recoverable amounts of isoprenoids. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein the cells produce increased amounts of isoprenoids compared to isoprenoid producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0125] In any of the aspects herein, the present invention provides recombinant cells, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and can be further engineered to modulate the activity of one or more of the following genes including ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*), glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*,

and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*) to improve carbon flux through the phosphoketolase pathway.

[0126] In some embodiments, the present invention provides recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, and (iii) is further engineered to modulate the activity of one or more genes to increases carbon flux through the phosphoketolase pathway, wherein the cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0127] In some embodiments, the present invention provides recombinant cells capable of producing of isopreniods, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, (iii) is further engineered to modulate the activity of one or more genes to increases carbon flux through the phosphoketolase pathway, and (iv) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein the cells produce increased amounts of isoprenoids compared to isoprenoid producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

General Techniques

[0128] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, "*Molecular Cloning: A Laboratory Manual*", second edition (Sambrook et al., 1989); "*Oligonucleotide Synthesis*" (M. J. Gait, ed., 1984); "*Animal Cell Culture*" (R. I. Freshney, ed., 1987); "*Methods in Enzymology*" (Academic Press, Inc.); "*Current Protocols in Molecular Biology*" (F. M. Ausubel et al., eds., 1987, and

periodic updates); "*PCR: The Polymerase Chain Reaction*", (Mullis et al., eds., 1994). Singleton et al., *Dictionary of Microbiology and Molecular Biology* 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

Definitions

[0129] The term "isoprene" refers to 2-methyl-1,3-butadiene (CAS# 78-79-5). It can be the direct and final volatile C5 hydrocarbon product from the elimination of pyrophosphate from 3,3-dimethylallyl diphosphate (DMAPP). It may not involve the linking or polymerization of IPP molecules to DMAPP molecules. The term "isoprene" is not generally intended to be limited to its method of production unless indicated otherwise herein.

[0130] As used herein, the term "polypeptides" includes polypeptides, proteins, peptides, fragments of polypeptides, and fusion polypeptides.

[0131] As used herein, an "isolated polypeptide" is not part of a library of polypeptides, such as a library of 2, 5, 10, 20, 50 or more different polypeptides and is separated from at least one component with which it occurs in nature. An isolated polypeptide can be obtained, for example, by expression of a recombinant nucleic acid encoding the polypeptide.

[0132] By "heterologous polypeptide" is meant a polypeptide encoded by a nucleic acid sequence derived from a different organism, species, or strain than the host cell. In some embodiments, a heterologous polypeptide is not identical to a wild-type polypeptide that is found in the same host cell in nature.

[0133] As used herein, a "nucleic acid" refers to two or more deoxyribonucleotides and/or ribonucleotides covalently joined together in either single or double-stranded form.

[0134] By "recombinant nucleic acid" is meant a nucleic acid of interest that is free of one or more nucleic acids (*e.g.*, genes) which, in the genome occurring in nature of the organism from which the nucleic acid of interest is derived, flank the nucleic acid of interest. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA, a genomic DNA fragment, or a

cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences.

[0135] By "heterologous nucleic acid" is meant a nucleic acid sequence derived from a different organism, species or strain than the host cell. In some embodiments, the heterologous nucleic acid is not identical to a wild-type nucleic acid that is found in the same host cell in nature. For example, a nucleic acid encoded by the phosphoketolase gene from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei* and used to transform an *E. coli* is a heterologous nucleic acid.

[0136] As used herein, the terms "phosphoketolase", "phosphoketolase enzyme" or "phosphoketolase polypeptide" are used interchangeably and refer to a polypeptide that converts 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate and/or converts fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. Generally, phosphoketolases act upon ketoses. In certain embodiments, the phosphoketolase polypeptide catalyzes the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate. In other embodiments, the phosphoketolase polypeptide catalyzes the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. In other embodiments, the phosphoketolase polypeptide catalyzes the conversion of sedoheptulose-7-phosphate to a product (e.g., ribose-5-phosphate) and acetyl phosphate.

[0137] As used herein, an "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid of interest. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. An expression control sequence can be "native" or heterologous. A native expression control sequence is derived from the same organism, species, or strain as the gene being expressed. A heterologous expression control sequence is derived from a different organism, species, or strain as the gene being expressed. An "inducible promoter" is a promoter that is active under environmental or developmental regulation.

[0138] By "operably linked" is meant a functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence, wherein

the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0139] As used herein, the terms "minimal medium" or "minimal media" refer to growth media containing the minimum nutrients possible for cell growth, generally without the presence of amino acids. Minimal medium typically contains: (1) a carbon source for bacterial growth; (2) various salts, which can vary among bacterial species and growing conditions; and (3) water. The carbon source can vary significantly, from simple sugars like glucose to more complex hydrolysates of other biomass, such as yeast extract, as discussed in more detail below. The salts generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the cells to synthesize proteins and nucleic acids. Minimal medium can also be supplemented with selective agents, such as antibiotics, to select for the maintenance of certain plasmids and the like. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent cells lacking the resistance from growing. Medium can be supplemented with other compounds as necessary to select for desired physiological or biochemical characteristics, such as particular amino acids and the like.

[0140] As used herein, the term "isoprenoid" refers to a large and diverse class of naturally-occurring class of organic compounds composed of two or more units of hydrocarbons, with each unit consisting of five carbon atoms arranged in a specific pattern. As used herein, "isoprene" is expressly excluded from the definition of "isoprenoid."

[0141] As used herein, the term "terpenoid" refers to a large and diverse class of organic molecules derived from five-carbon isoprenoid units assembled and modified in a variety of ways and classified in groups based on the number of isoprenoid units used in group members. Hemiterpenoids have one isoprenoid unit. Monoterpenoids have two isoprenoid units. Sesquiterpenoids have three isoprenoid units. Diterpenoids have four isoprene units. Sesterterpenoids have five isoprenoid units. Triterpenoids have six isoprenoid units. Tetraterpenoids have eight isoprenoid units. Polyterpenoids have more than eight isoprenoid units.

[0142] As used herein, "isoprenoid precursor" refers to any molecule that is used by organisms in the biosynthesis of terpenoids or isoprenoids. Non-limiting examples of isoprenoid

precursor molecules include, e.g., mevalonate (e.g., mevalonic acid (MVA)), isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP).

[0143] As used herein, the term "mass yield" refers to the mass of the product produced by the recombinant cells divided by the mass of the glucose consumed by the recombinant cells expressed as a percentage.

[0144] By "specific productivity," it is meant the mass of the product produced by the recombinant cell divided by the product of the time for production, the cell density, and the volume of the culture.

[0145] By "titer," it is meant the mass of the product produced by the recombinant cells divided by the volume of the culture.

[0146] As used herein, the term "cell productivity index (CPI)" refers to the mass of the product produced by the recombinant cells divided by the mass of the recombinant cells produced in the culture.

[0147] Unless defined otherwise herein, 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.

[0148] As used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

[0149] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

Recombinant cells expressing a phosphoketolase polypeptide and one or more polypeptides of the MVA pathway

[0150] The mevalonate-dependent biosynthetic pathway (MVA pathway) is a key metabolic pathway present in all higher eukaryotes and certain bacteria. In addition to being important for the production of molecules used in processes as diverse as protein prenylation, cell membrane maintenance, protein anchoring, and N-glycosylation, the mevalonate pathway provides a major source of the isoprenoid precursor molecules DMAPP and IPP, which serve as the basis for the biosynthesis of terpenes, terpenoids, isoprenoids, and isoprene.

[0151] The complete MVA pathway can be subdivided into two groups: an upper and lower pathway. In the upper portion of the MVA pathway, acetyl Co-A produced during cellular metabolism is converted to mevalonate via the actions of polypeptides having either: (a) (i) thiolase activity or (ii) acetoacetyl-CoA synthase activity, (b) HMG-CoA reductase, and (c) HMG-CoA synthase enzymatic activity. First, acetyl Co-A is converted to acetoacetyl CoA via the action of a thiolase or an acetoacetyl-CoA synthase (which utilizes acetyl-CoA and malonyl-CoA). Next, acetoacetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by the enzymatic action of HMG-CoA synthase. This Co-A derivative is reduced to mevalonate by HMG-CoA reductase, which is the rate-limiting step of the mevalonate pathway of isoprenoid production. In the lower MVA pathway, mevalonate is then converted into mevalonate-5-phosphate via the action of mevalonate kinase which is subsequently transformed into 5-diphosphomevalonate by the enzymatic activity of phosphomevalonate kinase. Finally, IPP is formed from 5-diphosphomevalonate by the activity of the enzyme mevalonate-5-pyrophosphate decarboxylase.

[0152] Thus, in certain embodiments, the recombinant cells of the present invention are recombinant cells having the ability to produce mevalonate, isoprenoid precursors, isoprene or isoprenoids via the MVA pathway wherein the recombinant cells comprise: (i) a heterologous gene encoding a phosphoketolase capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate, (ii) one or more heterologous genes encoding one or more MVA polypeptides, and (iii) one or more heterologous genes involved in mevalonate, isoprenoid precursor, or isoprene or isoprenoid biosynthesis that enables the synthesis of mevalonate, isoprenoid precursors, isoprene or isoprenoids from acetoacetyl-CoA in the host cell. In other embodiments, recombinant cells of the present invention are recombinant cells

having the ability to produce mevalonate, isoprenoid precursors, isoprene or isoprenoids wherein the recombinant cells comprise: (i) a heterologous gene encoding a phosphoketolase capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate, (ii) one or more heterologous genes encoding one or more MVA polypeptides, and (iii) one or more heterologous genes involved in mevalonate, isoprenoid precursors, isoprene or isoprenoid biosynthesis that enables the synthesis of produce mevalonate, isoprenoid precursors, isoprene or isoprenoids from acetoacetyl-CoA in the host cell.

Exemplary phosphoketolase polypeptides and nucleic acids

[0153] Phosphoketolase enzymes catalyze the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate and/or the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. In certain embodiments, the phosphoketolase enzyme is capable of catalyzing the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate. In other embodiments, the phosphoketolase enzyme is capable of catalyzing the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. In other embodiments, the phosphoketolase polypeptide catalyzes the conversion of sedoheptulose-7-phosphate to a product (e.g., ribose-5-phosphate) and acetyl phosphate. Thus, without being bound by theory, the expression of phosphoketolase as set forth herein can result in an increase in the amount of acetyl phosphate produced from a carbohydrate source. This acetyl phosphate can be converted into acetyl-CoA which can then be utilized by the enzymatic activities of the MVA pathway to produces mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids. Thus the amount of these compounds produced from a carbohydrate substrate may be increased. Alternatively, production of Acetyl-P and AcCoA can be increased without the increase being reflected in higher intracellular concentration. In certain embodiments, intracellular acetyl-P or acetyl-CoA concentrations will remain unchanged or even decrease, even though the phosphoketolase reaction is taking place.

[0154] Exemplary phosphoketolase nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a phosphoketolase polypeptide. Exemplary phosphoketolase polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein (See for example Figure 4). Additionally, Table 1

provides a non-limiting list of species and biochemical characteristics of certain exemplary phosphoketolases which may be utilized within embodiments of the invention.

[0155] Biochemical characteristics of exemplary phosphoketolases include, but are not limited to, protein expression, protein solubility, and activity. Phosphoketolases can also be selected on the basis of other characteristics, including, but not limited to, diversity amongst different types of organisms (e.g., gram positive bacteria, cyanobacteria, actinomyces), facultative low temperature aerobe, close relatives to a desired species (e.g., *E. coli*), and thermotolerance.

[0156] As provided herein, phosphoketolase activity can improve production of isoprenoid precursors (e.g., mevalonate), isoprene, and/or isoprenoids. Provided herein is a recombinant host comprising phosphoketolase wherein the cells display at least one property of interest to improve production of isoprenoid precursors (e.g., mevalonate), isoprene, and/or isoprenoids. In some aspects, at least one property of interest is selected from but not limited to the group consisting of specific productivity, yield, titer and cellular performance index.

[0157] In certain embodiments, suitable phosphoketolases for use herein include soluble phosphoketolases. Techniques for measuring protein solubility are well known in the art. Techniques for measuring protein solubility include those disclosed herein in the Examples. In some embodiments, a phosphoketolase for use herein includes those with a solubility of at least 20%. In some embodiments, phosphoketolase solubility is between about any of 5% to about 100%, between about 10% to about 100%, between about 15% to about 100%, between about 20% to about 100%, between about 25% to about 100%, between about 30% to about 100%, between about 35% to about 100%, between about 40% to about 100%, between about 45% to about 100%, between about 50% to about 100%, between about 55% to about 100%, between about 60% to about 100%, between about 65% to about 100%, between about 70% to about 100%, between about 75% to about 100%, between about 80% to about 100%, between about 85% to about 100%, or between about 90% to about 100%, In some embodiments, phosphoketolase solubility is between about 5% to about 100%. In some embodiments, solubility is between 5% and 100%. In some embodiments, phosphoketolase solubility is less than about any of 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 but no less than about 5%. In some embodiments, solubility is greater than about any of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95%.

[0158] Phosphoketolases with a desired kinetic characteristic increases the production of isoprene. Kinetic characteristics include, but are not limited to, specific activity, K_{cat}, K_i, and K_m. In some aspects, the k_{cat} is at least about 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.1, 8.2, 8.4, 8.6, 8.8, 9.0, 9.2, 9.4, 9.6, 9.8, 10.0, 10.2, 10.4, 10.6, 10.8, 11.0, 11.2, 11.4, 11.6, 11.8, 12.0, 12.2, 12.4, 12.6, 12.8, 13.0, 13.2, 13.4, 13.6, 13.8, 14.0, 14.2, 14.4, 14.6, 14.8, 15.0, 15.2, 15.4, 15.6, 15.8, 16.0, 16.2, 16.4, 16.6, 16.8, 17.0, 17.2, 17.4, 17.6, 17.8, 18.0, 18.2, 18.4, 18.6, 18.8, 19.0, 19.2, 19.4, 19.6, 19.8, 20.0, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, or 800.. In other aspects, the k_{cat} is at least about 0.2, 0.4, 0.6, 0.8, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.1, 8.2, 8.4, 8.6, 8.8, 9.0, 9.2, 9.4, 9.6, 9.8, 10.0, 10.2, 10.4, 10.6, 10.8, 11.0, 11.2, 11.4, 11.6, 11.8, 12.0, 12.2, 12.4, 12.6, 12.8, 13.0, 13.2, 13.4, 13.6, 13.8, 14.0, 14.2, 14.4, 14.6, 14.8, 15.0, 15.2, 15.4, 15.6, 15.8, 16.0, 16.2, 16.4, or 16.6.

[0159] In some aspects, the K_m is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, or 56. In other aspects, the k_m is at least about 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, or 22.

[0160] Properties of interest include, but are not limited to: increased intracellular activity, specific productivity, yield, and cellular performance index as compared to as compared to a recombinant cell that does not comprise the phosphoketolase polypeptide. In some embodiments, specific productivity increase at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6 7, 8, 9, 10 times or more. In one embodiment, specific productivity is about 40 mg/L/OD/hr. In some embodiments, yield increase of at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5 times or more. In other embodiments, MVA yield increase of at least

about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5 times or more. In other embodiments, isoprene yield increase of at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5 times or more. In other embodiments, cell performance index increase at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5 times or more. In other embodiments, intracellular activity increase at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10 times or more.

Table 1: Kinetic characteristics of phosphoketolases reported in the literature

Organism	Kinetic constants	Reference
Bifidobacterium lactis	K_m(F6P)=10 mM K_m(X5P)=55 mM k_{cat}(F6P)=8 s^{-1} k_{cat}(X5P)=42 s^{-1}	Meile et al.(2001) *J.Bacteriol.*, v. 183 (9), pp. 2929-2936
Bifidobacterium longum BB536	K_m(F6P) = 26 mM k_{cat} (F6P) = 39 s^{-1}	Grill et al. (1995) *Curr.Microbiol.*, v. 31, pp. 49-54
Bifidobacterium dentium ATCC27534	K_m(F6P) = 23 mM k_{cat} (F6P) = 33 s^{-1}	Grill et al. (1995) *Curr.Microbiol.*, v. 31, pp. 49-54
Bifidobacterium animalis ATCC 25527	K_m(F6P) = 11.5 mM k_{cat} (F6P) = 33 s^{-1}	Grill et al. (1995) *Curr.Microbiol.*, v. 31, pp. 49-54
Bifidobacterium globosum ATCC 25864	K_m(F6P) = 12.5 mM k_{cat} (F6P) = 40 s^{-1}	Grill et al. (1995) *Curr.Microbiol.*, v. 31, pp. 49-54
Lactobacillus plantarum	K_m(F6P)=24 mM K_m(X5P)=3.6 mM K_m(Pi)=2.9 mM (for F6P as a substrate) K_m(Pi)=7.5 mM (for X5P as a substrate) K_{is}^{E4P}=8 mM^1 (for F6P as a substrate) K_{ii}^{E4P}=4.4 mM^1 (for F6P as a substrate)	Yevenes and Frey (2008) *Bioorg.Chem.*, v. 36, pp. 121-127.

	k_{cat}(F6P)=2.7 s^{-1} k_{cat}(X5P)=6.1 s^{-1}	
Bifidobacterium breve	K_m(F6P)=9.7 mM K_mPi=1.2 mM (for F6P as a substrate) k_{cat} (F6P)=22.4 s^{-1} k_{cat} (X5P)=44.8 s^{-1}	Suzuki et al. (2010) *Acta Cryst.*, v. F66, pp. 941-943.
Lactobacillus paraplantarum C7	T_m = 45°C, pH_{opt}=7.0 K_m(F6P)=5.1 mM k_{cat}(F6P)=738 s^{-1}	Jeong et al. (2007) *J.Microbiol.Biotechnol.*, v. 17(5), pp. 822-829.
Leuconostoc oenos	K_m(F6P)=22 mM K_m(X5P)=1.6 mM k_{cat} (F6P)=0.2 s-1 k_{cat} (X5P)=3.5 s^{-1}	Veiga-da-Cunha et al. (1993) *J.Bacteriol.*, v. 175(13), pp. 3941-3948.

[0161] Other phosphoketolases that can be used include, but are not limited to, *B. longum, L. plantarum, C. acetobutylicum, L. reuteri, L. paraplantarum, R. palustris, Nostoc punctiforme, B. animalis, B. breve, G. vaginalis, E. gallinarum, M. paludis, Panteoa sp., R. aquatilis, N. punctiforme, S. avermetilis,* and *T. fusca.*

[0162] Standard methods can be used to determine whether a polypeptide has phosphoketolase peptide activity by measuring the ability of the peptide to convert D-fructose 6-phosphate or D-xylulose 5-phosphate into acetyl-P. Acetyl-P can then be converted into ferryl acetyl hydroxamate, which can be detected spectrophotometrically (Meile *et al.*, J. Bact. 183:2929-2936, 2001). Any polypeptide identified as having phosphoketolase peptide activity as described herein is suitable for use in the present invention. In some embodiments, the phosphoketolase polypeptide catalyzes the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate. In other embodiments, tha phosphoketolase polypeptide catalyzes the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate. In still other embodiments, the phosphoketolase polypeptide capable of catalyzing the conversion of sedoheptulose-7-phosphate to ribose-5-phosphate and acetyl

phosphate. In still other embodiments, the phosphoketolase polypeptide catalyzes the conversion of xylulose 5-phosphate to glyceraldehyde 3-phosphate and acetyl phosphate and/or the conversion of fructose 6-phosphate to erythrose 4-phosphate and acetyl phosphate and/or the conversion of sedoheptulose-7-phosphate to ribose-5-phosphate and acetyl phosphate.

[0163] Provided herein is a phosphoketolase isolated from a microorganism. In some aspects, a phosphoketolase isolated from the group consisting of a gram positive bacterium, a gram negative bacterium, an aerobic bacterium, an anaerobic bacterium, a thermophilic bacterium, a psychrophilic bacterium, a halophilic bacterium or a cyanobacterium. In some aspects, a phosphoketolase isolated from a fungi. In other apects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In other apects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea sp., Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In other apects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In any of the aspects described herein, a phosphoketolase nucleic acid can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to anyof the phosphoketolase nucleic acid sequences described herein. The phosphoketolase nucleic acid encoded by the *Lactobacillus reuteri* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:1. The phosphoketolase nucleic acid encoded by the *Bifidobacterium longum* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:3. The phosphoketolase nucleic acid encoded by the *Enterococcus gallinarum* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:17. The phosphoketolase nucleic acid

encoded by the *Nostoc punctiforme* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:18. The phosphoketolase nucleic acid encoded by the *Rhodopseudomonas palustris* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:19. The phosphoketolase nucleic acid encoded by the *Pantoea sp.* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:20. The phosphoketolase nucleic acid encoded by the *Mucilaginibacter paludis* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:21. The phosphoketolase nucleic acid encoded by the *Thermobifida fusca* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:22. The phosphoketolase nucleic acid encoded by the *Bifidobacterium breve* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:23. The phosphoketolase nucleic acid encoded by the *Rahnella aquatilis* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:24. The phosphoketolase nucleic acid encoded by the *Bifidobacterium animalis* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:25. The phosphoketolase nucleic acid encoded by the *Gardnerella vaginalis* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:26. The phosphoketolase nucleic acid encoded by the *Streptomyces avermitilis* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:27. The phosphoketolase nucleic acid encoded by the *Clostridium acetobutylicum* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:28. The phosphoketolase nucleic acid encoded by the *Lactobacillus paraplantarum* phosphoketolase gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:29.

[0164] In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus reuteri* phosphoketolase nucleic acid sequence SEQ ID NO:1. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium longum* phosphoketolase nucleic acid sequence SEQ ID NO:3. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Enterococcus gallinarum* phosphoketolase nucleic acid sequence SEQ ID NO:17. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Nostoc punctiforme* phosphoketolase nucleic acid sequence SEQ ID NO:18. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Rhodopseudomonas palustris* phosphoketolase nucleic acid sequence SEQ ID NO:19. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Pantoea sp.* phosphoketolase nucleic acid sequence SEQ ID NO:20. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Mucilaginibacter paludis* phosphoketolase nucleic acid sequence SEQ ID NO:21. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Thermobifida fusca* phosphoketolase nucleic acid sequence SEQ ID NO:22. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium breve* phosphoketolase nucleic acid sequence SEQ ID NO:23. In some embodiments, the phosphoketolase polypeptide can have at least about 99%,

98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Rahnella aquatilis* phosphoketolase nucleic acid sequence SEQ ID NO:24. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium animalis* phosphoketolase nucleic acid sequence SEQ ID NO:25. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Gardnerella vaginalis* phosphoketolase nucleic acid sequence SEQ ID NO:26. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Streptomyces avermitilis* phosphoketolase nucleic acid sequence SEQ ID NO:27. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Clostridium acetobutylicum* phosphoketolase nucleic acid sequence SEQ ID NO:28. In some embodiments, the phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus paraplantarum* phosphoketolase polypeptide can have nucleic acid sequence SEQ ID NO:29. In any of the aspects described herein, a phosphoketolase polypeptide can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to any of the phosphoketolase polypeptide encoded by any of phosphoketolase nucleic acid sequences described herein.

[0165] Additional examples of phosphoketolase enzymes which can be used herein are described in U.S. 7,785,858 and WO 2011/159853, which are incorporated by reference herein, especially with respect to all disclosure about phosphoketolase enzymes.

Upper MVA pathway polypeptides

[0166] The upper portion of the MVA pathway uses acetyl Co-A produced during cellular metabolism as the initial substrate for conversion to mevalonate via the actions of polypeptides having either: (a) (i) thiolase activity or (ii) acetoacetyl-CoA activity, (b) HMG-

CoA reductase, and (c) HMG-CoA synthase enzymatic activity. First, acetyl Co-A is converted to acetoacetyl CoA via the action of a thiolase or an acetoacetyl-CoA synthase (which utilizes acetyl-CoA and malonyl-CoA). Next, acetoacetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by the enzymatic action of HMG-CoA synthase. This Co-A derivative is reduced to mevalonate by HMG-CoA reductase, which is the rate-limiting step of the mevalonate pathway of isoprenoid production.

[0167] Non-limiting examples of upper MVA pathway polypeptides include acetyl-CoA acetyltransferase (AA-CoA thiolase) polypeptides, acetoacetyl-CoA synthase polypeptides, 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) polypeptides, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) polypeptides. Upper MVA pathway polypeptides can include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of an upper MVA pathway polypeptide. Exemplary upper MVA pathway nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of an upper MVA pathway polypeptide. Exemplary MVA pathway polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein. Thus, it is contemplated herein that any gene encoding an upper MVA pathway polypeptide can be used in the present invention.

[0168] In certain embodiments, various options of *mvaE* and *mvaS* genes from *L. grayi, E. faecium, E. gallinarum,* E. casseliflavus and/or E. faecalis alone or in combination with one or more other *mvaE* and *mvaS* genes encoding proteins from the upper MVA pathway are contemplated within the scope of the invention. In other embodiments, an acetoacetyl-CoA synthase gene is contemplated within the scope of the present invention in combination with one or more other genes encoding: (i) 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) polypeptides and 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) polypeptides. Thus, in certain aspects, any of the combinations of genes contemplated in can be expressed in recombinant cells in any of the ways described herein.

[0169] Additional non-limiting examples of upper MVA pathway polypeptides which can be used herein are described in International Patent Application Publication No. WO2009/076676; WO2010/003007 and WO2010/148150.

Genes encoding mvaE and mvaS polypeptides

[0170] In certain embodiments, various options of *mvaE* and *mvaS* genes from *L. grayi, E. faecium, E. gallinarum,* E. casseliflavus and/or E. faecalis alone or in combination with one or more other *mvaE* and *mvaS* genes encoding proteins from the upper MVA pathway are contemplated within the scope of the invention. In *L. grayi, E. faecium, E. gallinarum, E. casseliflavus,* and *E. faecalis*, the *mvaE* gene encodes a polypeptide that possesses both thiolase and HMG-CoA reductase activities. In fact, the *mvaE* gene product represented the first bifunctional enzyme of IPP biosynthesis found in eubacteria and the first example of HMG-CoA reductase fused to another protein in nature (Hedl, et al., *J Bacteriol.* 2002 April; 184(8): 2116–2122). The *mvaS* gene, on the other hand, encodes a polypeptide having an HMG-CoA synthase activity.

[0171] Accordingly, recombinant cells (e.g., *E. coli*) can be engineered to express one or more *mvaE* and *mvaS* genes from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus* and/or *E. faecalis,* to produce mevalonate. The one or more *mvaE* and *mvaS* genes can be expressed on a multicopy plasmid. The plasmid can be a high copy plasmid, a low copy plasmid, or a medium copy plasmid. Alternatively, the one or more *mvaE* and *mvaS* genes can be integrated into the host cell's chromosome. For both heterologous expression of the one or more *mvaE* and *mvaS* genes on a plasmid or as an integrated part of the host cell's chromosome, expression of the genes can be driven by either an inducible promoter or a constitutively expressing promoter. The promoter can be a strong driver of expression, it can be a weak driver of expression, or it can be a medium driver of expression of the one or more *mvaE* and *mvaS* genes.

Exemplary mvaE polypeptides and nucleic acids

[0172] The *mvaE* gene encodes a polypeptide that possesses both thiolase and HMG-CoA reductase activities. The thiolase activity of the polypeptide encoded by the *mvaE* gene converts acetyl Co-A to acetoacetyl CoA whereas the HMG-CoA reductase enzymatic activity of the polypeptide converts 3-hydroxy-3-methylglutaryl-CoA to mevalonate. Exemplary mvaE polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein that have at least one activity of a mvaE polypeptide.

[0173] Mutant mvaE polypeptides include those in which one or more amino acid residues have undergone an amino acid substitution while retaining mvaE polypeptide activity (*i.e.*, the ability to convert acetyl Co-A to acetoacetyl CoA as well as the ability to convert 3-hydroxy-3-methylglutaryl-CoA to mevalonate). The amino acid substitutions can be conservative or non-conservative and such substituted amino acid residues can or can not be one encoded by the genetic code. The standard twenty amino acid "alphabet" has been divided into chemical families based on similarity of their side chains. Those families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a chemically similar side chain (*i.e.*, replacing an amino acid having a basic side chain with another amino acid having a basic side chain). A "non-conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a chemically different side chain (*i.e.*, replacing an amino acid having a basic side chain with another amino acid having an aromatic side chain).

[0174] Amino acid substitutions in the mvaE polypeptide can be introduced to improve the functionality of the molecule. For example, amino acid substitutions that increase the binding affinity of the mvaE polypeptide for its substrate, or that improve its ability to convert acetyl Co-A to acetoacetyl CoA and/or the ability to convert 3-hydroxy-3-methylglutaryl-CoA to mevalonate can be introduced into the mvaE polypeptide. In some aspects, the mutant mvaE polypeptides contain one or more conservative amino acid substitutions.

[0175] In one aspect, mvaE proteins that are not degraded or less prone to degradation can be used for the production of mevalonate, isoprenoid precursors, isoprene, and/or isoprenoids. Examples of gene products of *mvaEs* that are not degraded or less prone to degradation which can be used include, but are not limited to, those from the organisms *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. faecalis*, and *L.* grayi. One of skill in the art can express mvaE protein in *E. coli* BL21 (DE3) and look for absence of fragments by any standard molecular biology techniques. For example, absence of fragments can be identified on Safestain

stained SDS-PAGE gels following His-tag mediated purification or when expressed in mevalonate, isoprene or isoprenoid producing *E. coli* BL21 using the methods of detection described herein.

[0176] Standard methods, such as those described in Hedl et al., (*J Bacteriol.* 2002, April; 184(8): 2116–2122) can be used to determine whether a polypeptide has mvaE activity, by measuring acetoacetyl-CoA thiolase as well as HMG-CoA reductase activity. In an exemplary assay, acetoacetyl-CoA thiolase activity is measured by spectrophotometer to monitor the change in absorbance at 302 nm that accompanies the formation or thiolysis of acetoacetyl-CoA. Standard assay conditions for each reaction to determine synthesis of acetoacetyl-CoA, are 1 mM acetyl-CoA, 10 mM $MgCl_2$, 50 mM Tris, pH 10.5 and the reaction is initiated by addition of enzyme. Assays can employ a final volume of 200 µl. For the assay, 1 enzyme unit (eu) represents the synthesis or thiolysis in 1 min of 1 µmol of acetoacetyl-CoA. In another exemplary assay, of HMG-CoA reductase activity can be monitored by spectrophotometer by the appearance or disappearance of NADP(H) at 340 nm. Standard assay conditions for each reaction measured to show reductive deacylation of HMG-CoA to mevalonate are 0.4 mM NADPH, 1.0 mM (*R*,*S*)-HMG-CoA, 100 mM KCl, and 100 mM K_xPO_4, pH 6.5. Assays employ a final volume of 200 µl. Reactions are initiated by adding the enzyme. For the assay, 1 eu represents the turnover, in 1 min, of 1 µmol of NADP(H). This corresponds to the turnover of 0.5 µmol of HMG-CoA or mevalonate.

[0177] Alternatively, production of mevalonate in recombinant cells can be measured by, without limitation, gas chromatography (see U.S. Patent Application Publication No.: US 2005/0287655 A1) or HPLC (See U.S. Patent Application Publication No.: 2011/0159557 A1). As an exemplary assay, cultures can be inoculated in shake tubes containing LB broth supplemented with one or more antibiotics and incubated for 14h at 34°C at 250 rpm. Next, cultures can be diluted into well plates containing TM3 media supplemented with 1% Glucose, 0.1% yeast extract, and 200 µM IPTG to final OD of 0.2. The plate are then sealed with a Breath Easier membrane (Diversified Biotech) and incubated at 34°C in a shaker/incubator at 600 rpm for 24 hours. 1 mL of each culture is then centrifuged at 3,000 x g for 5 min. Supernatant is then added to 20% sulfuric acid and incubated on ice for 5 min. The mixture is then centrifuged for 5 min at 3000 x g and the supernatant was collected for HPLC analysis. The concentration of mevalonate in samples is determined by comparison to a standard curve of

mevalonate (Sigma). The glucose concentration can additionally be measured by performing a glucose oxidase assay according to any method known in the art. Using HPLC, levels of mevalonate can be quantified by comparing the refractive index response of each sample versus a calibration curve generated by running various mevalonate containing solutions of known concentration.

[0178] Exemplary mvaE nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a mvaE polypeptide. Exemplary mvaE polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein. Exemplary mvaE nucleic acids include, for example, mvaE nucleic acids isolated from *Listeria grayi_DSM 20601,* Enterococcus *faecium, Enterococcus gallinarum EG2, Enterococcus faecalis,* and/or *Enterococcus casseliflavus.* The mvaE nucleic acid encoded by the *Listeria grayi_DSM 20601 mvaE* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:6. The mvaE nucleic acid encoded by the *Enterococcus faecium mvaE* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:7. The mvaE nucleic acid encoded by the *Enterococcus gallinarum EG2 mvaE* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:8. The mvaE nucleic acid encoded by the *Enterococcus casseliflavus mvaE* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:9. The mvaE nucleic acid encoded by the *Enterococcus faecalis mvaE* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to the *mvaE* gene previously disclosed in *E. coli* to produce mevalonate (see US 2005/0287655 A1; Tabata, K. and Hashimoto,S.-I. *Biotechnology Letters* 26: 1487–1491, 2004).

[0179] The mvaE nucleic acid can be expressed in a recombinant cell on a multicopy plasmid. The plasmid can be a high copy plasmid, a low copy plasmid, or a medium copy plasmid. Alternatively, the mvaE nucleic acid can be integrated into the host cell's chromosome. For both heterologous expression of an mvaE nucleic acid on a plasmid or as an

integrated part of the host cell's chromosome, expression of the nucleic acid can be driven by either an inducible promoter or a constitutively expressing promoter. The promoter can be a strong driver of expression, it can be a weak driver of expression, or it can be a medium driver of expression of the mvaE nucleic acid.

Exemplary mvaS polypeptides and nucleic acids

[0180] The *mvaS* gene encodes a polypeptide that possesses HMG-CoA synthase activity. This polypeptide can convert acetoacetyl CoA to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Exemplary mvaS polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein that have at least one activity of a mvaS polypeptide.

[0181] Mutant mvaS polypeptides include those in which one or more amino acid residues have undergone an amino acid substitution while retaining mvaS polypeptide activity (*i.e.*, the ability to convert acetoacetyl CoA to 3-hydroxy-3-methylglutaryl-CoA). Amino acid substitutions in the mvaS polypeptide can be introduced to improve the functionality of the molecule. For example, amino acid substitutions that increase the binding affinity of the mvaS polypeptide for its substrate, or that improve its ability to convert acetoacetyl CoA to 3-hydroxy-3-methylglutaryl-CoA can be introduced into the mvaS polypeptide. In some aspects, the mutant mvaS polypeptides contain one or more conservative amino acid substitutions.

[0182] Standard methods, such as those described in Quant et al. (*Biochem J.,* 1989, 262:159-164), can be used to determine whether a polypeptide has mvaS activity, by measuring HMG-CoA synthase activity. In an exemplary assay, HMG-CoA synthase activity can be assayed by spectrophotometrically measuring the disappearance of the enol form of acetoacetyl-CoA by monitoring the change of absorbance at 303 nm. A standard 1 ml assay system containing 50 mm-Tris/HCl, pH 8.0, 10 mM-MgCl2 and 0.2 mM-dithiothreitol at 30 °C; 5 mM-acetyl phosphate, 10,M-acetoacetyl- CoA and 5 µ1 samples of extracts can be added, followed by simultaneous addition of acetyl-CoA (100 µM) and 10 units of PTA. HMG-CoA synthase activity is then measured as the difference in the rate before and after acetyl-CoA addition. The absorption coefficient of acetoacetyl-CoA under the conditions used (pH 8.0, 10 mM-$MgCl_2$), is

12.2 x 10^3 M^{-1} cm^{-1}. By definition, 1 unit of enzyme activity causes 1 µmol of acetoacetyl-CoA to be transformed per minute.

[0183] Alternatively, production of mevalonate in recombinant cells can be measured by, without limitation, gas chromatography (see U.S. Patent Application Publication No.: US 2005/0287655 A1) or HPLC (See U.S. Patent Application Publication No.: 2011/0159557 A1). As an exemplary assay, cultures can be inoculated in shake tubes containing LB broth supplemented with one or more antibiotics and incubated for 14h at 34°C at 250 rpm. Next, cultures can be diluted into well plates containing TM3 media supplemented with 1% Glucose, 0.1% yeast extract, and 200 µM IPTG to final OD of 0.2. The plate are then sealed with a Breath Easier membrane (Diversified Biotech) and incubated at 34°C in a shaker/incubator at 600 rpm for 24 hours. 1 mL of each culture is then centrifuged at 3,000 x g for 5 min. Supernatant is then added to 20% sulfuric acid and incubated on ice for 5 min. The mixture is then centrifuged for 5 min at 3000 x g and the supernatant was collected for HPLC analysis. The concentration of mevalonate in samples is determined by comparison to a standard curve of mevalonate (Sigma). The glucose concentration can additionally be measured by performing a glucose oxidase assay according to any method known in the art. Using HPLC, levels of mevalonate can be quantified by comparing the refractive index response of each sample versus a calibration curve generated by running various mevonate containing solutions of known concentration.

[0184] Exemplary mvaS nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a mvaS polypeptide. Exemplary mvaS polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein. Exemplary mvaS nucleic acids include, for example, mvaS nucleic acids isolated from *Listeria grayi_DSM 20601, Enterococcus faecium, Enterococcus gallinarum EG2, Enterococcus faecalis,* and/or *Enterococcus casseliflavus.* The mvaS nucleic acid encoded by the *Listeria grayi_DSM 20601 mvaS* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:10. The mvaS nucleic acid encoded by the *Enterococcus faecium mvaS* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity

to SEQ ID NO:11. The mvaS nucleic acid encoded by the *Enterococcus gallinarum EG2 mvaS* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:12. The mvaS nucleic acid encoded by the *Enterococcus casseliflavus mvaS* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to SEQ ID NO:13. The mvaS nucleic acid encoded by the *Enterococcus faecalis mvaS* gene can have at least about 99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, or 85% sequence identity to to the *mvaE* gene previously disclosed in *E. coli* to produce mevalonate (see US 2005/0287655 A1; Tabata, K. and Hashimoto,S.-I. *Biotechnology Letters* 26: 1487–1491, 2004).

[0185] The mvaS nucleic acid can be expressed in a recombinant cell on a multicopy plasmid. The plasmid can be a high copy plasmid, a low copy plasmid, or a medium copy plasmid. Alternatively, the mvaS nucleic acid can be integrated into the host cell's chromosome. For both heterologous expression of an mvaS nucleic acid on a plasmid or as an integrated part of the host cell's chromosome, expression of the nucleic acid can be driven by either an inducible promoter or a constitutively expressing promoter. The promoter can be a strong driver of expression, it can be a weak driver of expression, or it can be a medium driver of expression of the mvaS nucleic acid.

Acetoacetyl-CoA Synthase Gene

[0186] The acetoacetyl-CoA synthase gene (aka *nphT7*) is a gene encoding an enzyme having the activity of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA and having minimal activity (e.g., no activity) of synthesizing acetoacetyl-CoA from two acetyl-CoA molecules. *See, e.g.,* Okamura et al., *PNAS* Vol 107, No. 25, pp. 11265-11270 (2010), the contents of which are expressly incorporated herein for teaching about nphT7. An acetoacetyl-CoA synthase gene from an actinomycete of the genus *Streptomyces* CL190 strain was described in JP Patent Publication (Kokai) No. 2008-61506 A and US2010/0285549. Acetoacetyl-CoA synthase can also be referred to as acetyl CoA:malonyl CoA acyltransferase. A representative acetoacetyl-CoA synthase (or acetyl CoA:malonyl CoA acyltransferase) that can be used is Genbank AB540131.1.

[0187] In any of the aspects or embodiments described herein, an enzyme that has the ability to synthesize acetoacetyl-CoA from malonyl-CoA and acetyl-CoA can be used. Non-limiting examples of such an enzyme are described herein. In certain embodiments described herein, an acetoacetyl-CoA synthase gene derived from an actinomycete of the genus *Streptomyces* having the activity of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA can be used. An example of such an acetoacetyl-CoA synthase gene is the gene encoding a protein having the amino. Such a protein having the amino acid sequence of SEQ ID NO:5 corresponds to an acetoacetyl-CoA synthase having activity of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA and having no activity of synthesizing acetoacetyl-CoA from two acetyl-CoA molecules.

[0188] In one embodiment, the gene encoding a protein having the amino acid sequence of SEQ ID NO:5 can be obtained by a nucleic acid amplification method (e.g., PCR) with the use of genomic DNA obtained from an actinomycete of the *Streptomyces* sp. CL190 strain as a template and a pair of primers that can be designed with reference to JP Patent Publication (Kokai) No. 2008-61506 A.

[0189] As described herein, an acetoacetyl-CoA synthase gene for use in the present invention is not limited to a gene encoding a protein having the amino acid sequence of SEQ ID NO:5 from an actinomycete of the *Streptomyces* sp. CL190 strain. Any gene encoding a protein having the ability to synthesize acetoacetyl-CoA from malonyl-CoA and acetyl-CoA and which does not synthesize acetoacetyl-CoA from two acetyl-CoA molecules can be used in the presently described methods. In certain embodiments, the acetoacetyl-CoA synthase gene can be a gene encoding a protein having an amino acid sequence with high similarity or substantially identical to the amino acid sequence of SEQ ID NO:5 and having the function of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA. The expression "highly similar" or "substantially identical" refers to, for example, at least about 80% identity, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99% identity. As used above, the identity value corresponds to the percentage of identity between amino acid residues in a different amino acid sequence and the amino acid sequence of SEQ ID NO:5, which is calculated by performing alignment of the amino acid sequence of SEQ

ID NO:5 and the different amino acid sequence with the use of a program for searching for a sequence similarity.

[0190] In other embodiments, the acetoacetyl-CoA synthase gene may be a gene encoding a protein having an amino acid sequence derived from the amino acid sequence of SEQ ID NO:5 by substitution, deletion, addition, or insertion of 1 or more amino acid(s) and having the function of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA. Herein, the expression "more amino acids" refers to, for example, 2 to 30 amino acids, preferably 2 to 20 amino acids, more preferably 2 to 10 amino acids, and most preferably 2 to 5 amino acids.

[0191] In still other embodiments, the acetoacetyl-CoA synthase gene may consist of a polynucleotide capable of hybridizing to a portion or the entirety of a polynucleotide having a nucleotide sequence complementary to the nucleotide sequence encoding the amino acid sequence of SEQ ID NO:5 under stringent conditions and capable of encoding a protein having the function of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA. Herein, hybridization under stringent conditions corresponds to maintenance of binding under conditions of washing at 60 $^{\circ}$C two times SSC. Hybridization can be carried out by conventionally known methods such as the method described in J. Sambrook et al. Molecular Cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory (2001).

[0192] As described herein, a gene encoding an acetoacetyl-CoA synthase having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:5 can be isolated from potentially any organism, for example, an actinomycete that is not obtained from the *Streptomyces* sp. CL190 strain. In addition, acetoacetyl-CoA synthase genes for use herein can be obtained by modifying a polynucleotide encoding the amino acid sequence of SEQ ID NO:5 by a method known in the art. Mutagenesis of a nucleotide sequence can be carried out by a known method such as the Kunkel method or the gapped duplex method or by a method similar to either thereof. For instance, mutagenesis may be carried out with the use of a mutagenesis kit (e.g., product names; Mutant-K and Mutant-G (TAKARA Bio)) for site-specific mutagenesis, product name; an LA PCR in vitro Mutagenesis series kit (TAKARA Bio), and the like.

[0193] The activity of an acetoacetyl-CoA synthase having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:5 can be evaluated as described below.

Specifically, a gene encoding a protein to be evaluated is first introduced into a host cell such that the gene can be expressed therein, followed by purification of the protein by a technique such as chromatography. Malonyl-CoA and acetyl-CoA are added as substrates to a buffer containing the obtained protein to be evaluated, followed by, for example, incubation at a desired temperature (e.g., 10°C to 60°C). After the completion of reaction, the amount of substrate lost and/or the amount of product (acetoacetyl-CoA) produced are determined. Thus, it is possible to evaluate whether or not the protein being tested has the function of synthesizing acetoacetyl-CoA from malonyl-CoA and acetyl-CoA and to evaluate the degree of synthesis. In such case, it is possible to examine whether or not the protein has the activity of synthesizing acetoacetyl-CoA from two acetyl-CoA molecules by adding acetyl-CoA alone as a substrate to a buffer containing the obtained protein to be evaluated and determining the amount of substrate lost and/or the amount of product produced in a similar manner.

Recombinant cells capable of increased production of mevalonate

[0194] The recombinant cells (e.g., recombinant bacterial cells) described herein can produce mevalonate at an amount and/or concentration greater than that of the same cells without any manipulation to the various enzymatic pathways described herein. Thus, the recombinant cells (e.g., bacterial cells) that have been engineered for modulation in the various pathways described herein are useful in the enhance production of mevalonate.

[0195] Accordingly, in certain aspects, the invention provides recombinant cells capable of enhanced production of mevalonate, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein the cells produce increased amounts of mevalonate compared to cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0196] In certain aspects, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis* dassonvillei. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more

copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other aspects, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other aspects, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0197] In one embodiment, the recombinant cells further comprise one or more copies of a heterologous nucleic acid encoding mvaE and mvaS polypeptides from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis.* In another embodiment, the recombinant cells further comprise an acetoacetyl-CoA synthase and one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway.

[0198] In one embodiment, the recombinant cells can be further engineered to increase the activity of one or more of the following genes selected from the group consisting of ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, the recombinant cells can be further engineered to decrease the activity of one or more genes of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

[0199] In one aspect, the recombinant cells described herein can produce mevalonate at a higher volumetric productivity than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding a polypeptide having phosphoketolase activity. In certain embodiments, the recombinant cell can produce greater than 2.00 g/L/hr of mevalonate. Alternatively, the recombinant cells can produce greater than about 1.0 g/L/hr, 1.2 g/L/hr, 1.4 g/L/hr, 1.6 g/L/hr, 1.8 g/L/hr, 2.0 g/L/hr, 2.2 g/L/hr, 2.4 g/L/hr, 2.6 g/L/hr , 2.8 g/L/hr, 3.0 g/L/hr, 3.2 g/L/hr, 3.4 g/L/hr, 3.6 g/L/hr , 3.8 g/L/hr , 4.0g/L/hr. 4.2g/L/hr, 4.4 g/L/hr, 4.6 g/L/hr, 4.8g/L/hr, 5.0g/L/hr, 5.2 g/L/hr, 5.4 g/L/hr, 5.6 g/L/hr, 5.8g/L/hr, 6.0 g/L/hr of mevalonate, inclusive, as well as any numerical value in between these numbers.

[0200] In one aspect, the recombinant cells described herein can produce mevalonate at a higher titer than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding a polypeptide having phosphoketolase activity. These recombinant cells can produce greater than about 100 g/L peak titer of mevalonate after 48 hours of fermentation. Alternatively, the recombinant cells can produce greater than about 50 g/L, 60 g/L, 70 g/L, 80 g/L, 90 g/L, 100 g/L, 110 g/L, 120 g/L, 130 g/L, 140 g/L, 150 g/L, 160 g/L, 170 g/L, 180 g/L, 190 g/L, 200 g/L, 210 g/L, 220 g/L, 230 g/L, 240 g/L, 250 g/L, 260 g/L, 270 g/L, 280 g/L, 290 g/L, 300 g/L peak titer of mevalonate after 48 hours of fermentation, inclusive, as well as any numerical value in between these numbers.

[0201] In other embodiments, the recombinant cells described herein further comprise one or more mutations which increase carbon flux towards the MVA pathway and can thus produce higher titers of mevalonate in comparision to cells which have not been similarly

engineered. In such embodiments, the recombinant cells described herein produce mevalonate at a higher peak titer than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding phosphoketolase polypeptide having phosphoketolase activity. In one embodiment, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, the recombinant cells can be further further engineered to decrease the activity of one or more genes of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

[0202] In one aspect, the recombinant cells described herein can produce mevalonate at a higher cell productivity index (CPI) for mevalonate than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding a polypeptide having phosphoketolase activity. The recombinant cells can have a CPI for mevalonate of at least about 3.0 (g/g). Alternatively, the recombinant cells can have a CPI for mevalonate of at least about 1 (g/g), 2 (g/g), 3 (g/g), 4 (g/g), 5 (g/g), 6 (g/g), 7 (g/g), 8 (g/g), 9 (g/g), 10 (g/g), 11 (g/g), 12 (g/g), 13 (g/g), 14 (g/g), 15 (g/g), 20 (g/g), 25 (g/g), or 30 (g/g) inclusive, as well as any numerical value in between these numbers.

[0203] In certain embodiments, the recombinant cells described herein further comprise one or more mutations which increase carbon flux towards the MVA pathway which results in a higher cell productivity index (CPI) for mevalonate in comparision to cells which have not been similarly engineered. Additionally, the recombinant cells described herein have a higher CPI than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding phosphoketolase polypeptide having phosphoketolase activity. In one embodiment, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, these recombinant cells can be further engineered to decrease the activity of one or more genes of the

following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

[0204] Additionally, the cells described herein have a higher mass yield of mevalonate from glucose than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding phosphoketolase polypeptide having phosphoketolase activity. The recombinant cells can produce a mass yield of mevalonate from glucose of at least about 28%. Alternatively, the recombinant cells can produce a mass yield of mevalonate from glucose of at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or 55%, inclusive, as well as any numerical value in between these numbers.

[0205] In certain embodiments, the recombinant cells described herein further comprise one or more mutations which increase carbon flux towards the MVA pathway which results in a higher mass yield of mevalonate in comparision to cells which have not been similarly engineered. Additionally, the recombinant cells described herein have a higher mass yield of mevalonate than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding phosphoketolase polypeptide having phosphoketolase activity. In one embodiment, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of rribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, these recombinant cells can be further engineered to decrease the activity of one or more genes of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

[0206] In one aspect, the recombinant cells described herein produce mevalonate while accumulating less acetate in the fermentation broth as compared to the same cells lacking one or more copies of a heterologous nucleic acid encoding a polypeptide having phosphoketolase activity. The recombinant cells can produce increased levels of mevalonate while accumulating

less than 4.5 g/L of acetate in the fermentation broth over a 48 hr fermentation. Alternatively, the recombinant cells can produce increased levels of mevalonate while accumulating less than about 8.0 g/L, 7.5 g/L, 7.0 g/L, 6.5 g/L, 6.0 g/L, 5.5 g/L, 5.0 g/L, 4.5 g/L, 4.0 g/L, 3.5 g/L, 3.0 g/L, 2.5 g/L, 2.0 g/L, or 1.5 g/L, of acetate in the fermentation broth over a 48 hr fermentation inclusive, as well as any numerical value in between these numbers. In certain embodiments, the decreased accumulation of acetate in the fermentation broth can improve cell viability during the fermentation run.

[0207] In certain embodiments, the recombinant cells described herein further comprise one or more mutations which increase carbon flux towards the MVA pathway which results increased levels of mevalonate while accumulating less acetate in the fermentation broth in comparision to cells which have not been similarly engineered. In certain embodiments, the decreased accumulation of acetate in the fermentation broth can improve cell viability during the fermentation run.

Methods of using recombinant recombinant cells to produce increased amounts of mevalonate

[0208] Also provided herein are methods for the production of mevalonate. In some aspects, the method for producing mevalonate comprises: (a) culturing a composition comprising recombinant cells which have been engineered to increase carbon flux as described herein (including any of the recombinant cells described above), or progeny thereof, capable of producing mevalonate; and (b) producing mevalonate. In some aspects, the method of producing mevalonate comprises the steps of culturing any of the recombinant cells described herein under conditions suitable for the production of mevalonate and allowing the recombinant cells to produce mevalonate. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

[0209] As described herein, the methods of producing mevalonate comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously express a phosphoketolase polypeptide, wherein the cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides; and (b) producing mevalonate. In certain embodiment, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces*

griseus, and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* Additionally, the recombinant cells can produce mevalonate in concentrations greater than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides, when the cells are cultured in minimal medium. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding an phosphoketolase polypeptide from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei* is a heterologous nucleic acid that is integrated into the host cell's chromosome.

[0210] In certain embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In other embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a

heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* Additionally, the recombinant cells can produce mevalonate in concentrations greater than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides, when the cells are cultured in minimal medium. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding an phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* is a heterologous nucleic acid that is integrated into the host cell's chromosome.

[0211] The instant methods for the production of mevalonate produce can produce mevalonate using cells having a volumetric productivity of greater than 2.00 g/L/hr of mevalonate. Alternatively, the recombinant cells can produce greater than about 1.0 g/L/hr, 1.2 g/L/hr, 1.4 g/L/hr, 1.6 g/L/hr, 1.8 g/L/hr, 2.0 g/L/hr, 2.2 g/L/hr, 2.4 g/L/hr, 2.6 g/L/hr , 2.8 g/L/hr, 3.0 g/L/hr, 3.2 g/L/hr, 3.4 g/L/hr, 3.6 g/L/hr , 3.8 g/L/hr , 4.0g/L/hr. 4.2g/L/hr, 4.4 g/L/hr, 4.6 g/L/hr, 4.8g/L/hr, 5.0g/L/hr, 5.2 g/L/hr, 5.4 g/L/hr, 5.6 g/L/hr, 5.8g/L/hr, 6.0 g/L/hr of mevalonate, inclusive, as well as any numerical value in between these numbers. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

[0212] In other embodiments, the methods of producing mevalonate can comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously express a phosphoketolase polypeptide, wherein the cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides; and (b) producing mevalonate, wherein the recombinant cells produce mevalonate with a higher peak titer after 48 hours of fermentation than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment,

the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0213] The instant methods for the production of mevalonate can produce mevalonate using cells that can produce a peak titer of greater than about 100 g/L peak titer of mevalonate after 48 hours of fermentation. Alternatively, the recombinant cells can produce greater than about 50 g/L, 60 g/L, 70 g/L, 80 g/L, 90 g/L, 100 g/L, 110 g/L, 120 g/L, 130 g/L, 140 g/L, 150 g/L, 160 g/L, 170 g/L, 180 g/L, 190 g/L, 200 g/L, 210 g/L, 220 g/L, 230 g/L, 240 g/L, 250 g/L, 260 g/L, 270 g/L, 280 g/L, 290 g/L, 300 g/L peak titer of mevalonate after 48 hours of fermentation, inclusive, as well as any numerical value in between these numbers. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

[0214] In other embodiments, the methods of producing mevalonate can comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously express a phosphoketolase polypeptide, wherein the cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides; and (b) producing mevalonate, wherein the recombinant cells have a CPI for mevalonate higher than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous

nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*. In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*.

[0215] The instant methods for the production of mevalonate can produce mevalonate using cells with a CPI for mevalonate of at least about 3.0 (g/g). Alternatively, the recombinant cells can have a CPI for mevalonate of at least about 1 (g/g), 2 (g/g), 3 (g/g), 4 (g/g), 5 (g/g), 6 (g/g), 7 (g/g), 8 (g/g), 9 (g/g), 10 (g/g), 11 (g/g), 12 (g/g), 13 (g/g), 14 (g/g), 15 (g/g), 20 (g/g), 25 (g/g), or 30 (g/g) inclusive, as well as any numerical value in between these numbers. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

[0216] In certain embodiments, the methods of producing mevalonate can comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously express a phosphoketolase polypeptide, wherein the cells heterologously express

one or more copies of a gene encoding a phosphoketolase polypeptide along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides; and (b) producing mevalonate, wherein the recombinant cells display decreased oxygen uptake rate (OUR) as compared to that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide. In certain embodiments, the recombinant cells expressing one or more heterologous copies of a gene encoding an phosphoketolase polypeptide display up to 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold or 7-fold decrease in OUR as compared to recombinant cells that do not express a phosphoketolase.

[0217] Provided herein are methods of using any of the cells described above for enhanced mevalonate production. The production of mevalonate by the cells can be enhanced by the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or*

Thermobifida fusca. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*.

[0218] The production of mevalonate can be enhanced by about 1,000,000 folds (*e.g.*, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of mevalonate by mevalonate-producing cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. In certain embodiments described herein, the host cells have been further engineered increased carbon flux to MVA production. In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum*. In another embodiment, the recombinant cells comprise one or more copies of a

heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0219] In other aspects, the methods described herein can provide for the enhanced production of mevalonate can by at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 50 folds, 100 folds, 200 folds, 500 folds, 1000 folds, 2000 folds, 5000 folds, 10,000 folds, 20,000 folds, 50,000 folds, 100,000 folds, 200,000 folds, 500,000 folds, or 1,000,000 folds compared to the production of mevalonate by mevalonate-producing cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. In certain embodiments described herein, the host cells have been further engineered increased carbon flux to MVA production.

[0220] In addition, more specific cell culture conditions can be used to culture the cells in the methods described herein. For example, in some aspects, the method for the production of mevalonate comprises the steps of (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously have a phosphoketolase gene in minimal medium at 34°C, wherein the recombinant cells heterologously express one or more copies of a heterologous gene encoding a phosphoketolase polypeptide on a low to medium copy plasmid and under the control of a strong promoter; and (b) producing mevalonate. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from

Nocardiopsis dassonvillei. In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

Recombinant Cells Capable of Producing Isoprene

[0221] Isoprene (2-methyl-1,3-butadiene) is an important organic compound used in a wide array of applications. For instance, isoprene is employed as an intermediate or a starting material in the synthesis of numerous chemical compositions and polymers, including in the production of synthetic rubber. Isoprene is also an important biological material that is synthesized naturally by many plants and animals.

[0222] Isoprene is produced from DMAPP by the enzymatic action of isoprene synthase. Therefore, without being bound to theory, it is thought that increasing the cellular production of mevalonate in recombinant cells by any of the compositions and methods described above will likewise result in the production of higher amounts of isoprene. Increasing the molar yield of mevalonate production from glucose translates into higher molar yields of isoprenoid precursors, isoprene and/or isoprenoids produced from glucose when combined with appropriate enzymatic activity levels of mevalonate kinase, phosphomevalonate kinase,

diphosphomevalonate decarboxylase, isopentenyl diphosphate isomerase (e.g., the lower MVA pathway) and other appropriate enzymes for isoprene and isoprenoid production.

[0223] As described herein, the present invention provides recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway (i.e., the upper MVA pathway and the lower MVA pathway) and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells are capable of producing recoverable amounts of isoprene. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0224] Production of isoprene can also be made by using any of the recombinant host cells described herein further comprising one or more of the enzymatic pathways manipulations wherein enzyme activity is modulated to increase carbon flow towards mevalonate production. The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flow to mevalonate production can be used to produce isoprene. In one embodiment, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of rribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, these recombinant cells can be further engineered to decrease the activity of one or more genes of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

Nucleic acids encoding polypeptides of the lower MVA pathway

[0225] In some aspects of the invention, the cells described in any of the compositions or methods described herein further comprise one or more nucleic acids encoding a lower mevalonate (MVA) pathway polypeptide(s). In some aspects, the lower MVA pathway polypeptide is an endogenous polypeptide. In some aspects, the endogenous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a constitutive promoter. In some aspects, the endogenous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to an inducible promoter. In some aspects, the endogenous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a strong promoter. In a particular aspect, the cells are engineered to over-express the endogenous lower MVA pathway polypeptide relative to wild-type cells. In some aspects, the endogenous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a weak promoter.

[0226] The lower mevalonate biosynthetic pathway comprises mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and diphosphomevalonte decarboxylase (MVD). In some aspects, the lower MVA pathway can further comprise isopentenyl diphosphate isomerase (IDI). Cells provided herein can comprise at least one nucleic acid encoding isoprene synthase, one or more upper MVA pathway polypeptides, and/or one or more lower MVA pathway polypeptides. Polypeptides of the lower MVA pathway can be any enzyme (a) that phosphorylates mevalonate to mevalonate 5-phosphate; (b) that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (c) that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate. More particularly, the enzyme that phosphorylates mevalonate to mevalonate 5-phosphate can be from the group consisting of *M. mazei* mevalonate kinase, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, *yeast* mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, *Streptomyces* mevalonate kinase polypeptide, *Streptomyces CL190* mevalonate kinase polypeptide, and *M. Burtonii* mevalonate kinase polypeptide. In another aspect, the enzyme that phosphorylates mevalonate to mevalonate 5-phosphate is *M. mazei* mevalonate kinase.

[0227] In some aspects, the lower MVA pathway polypeptide is a heterologous polypeptide. In some aspects, the cells comprise more than one copy of a heterologous nucleic

acid encoding a lower MVA pathway polypeptide. In some aspects, the heterologous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a constitutive promoter. In some aspects, the heterologous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to an inducible promoter. In some aspects, the heterologous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a strong promoter. In some aspects, the heterologous nucleic acid encoding a lower MVA pathway polypeptide is operably linked to a weak promoter. In some aspects, the heterologous lower MVA pathway polypeptide is a polypeptide from *Saccharomyces cerevisiae, Enterococcus faecalis,* or *Methanosarcina mazei.*

[0228] The nucleic acids encoding a lower MVA pathway polypeptide(s) can be integrated into a genome of the cells or can be stably expressed in the cells. The nucleic acids encoding a lower MVA pathway polypeptide(s) can additionally be on a vector.

[0229] Exemplary lower MVA pathway polypeptides are also provided below: (i) mevalonate kinase (MVK); (ii) phosphomevalonate kinase (PMK); (iii) diphosphomevalonate decarboxylase (MVD); and (iv) isopentenyl diphosphate isomerase (IDI). In particular, the lower MVK polypeptide can be from the genus *Methanosarcina* and, more specifically, the lower MVK polypeptide can be from Methanosarcina *mazei.* In some embodiments, the lower MVK polypeptide can be from *M. burtonii.* Additional examples of lower MVA pathway polypeptides can be found in U.S. Patent Application Publication 2010/0086978 the contents of which are expressly incorporated herein by reference in their entirety with respect to lower MVK pathway polypeptides and lower MVK pathway polypeptide variant.

[0230] Lower MVA pathway polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of a lower MVA pathway polypeptide. Exemplary lower MVA pathway nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a lower MVA pathway polypeptide. Exemplary lower MVA pathway polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein. In addition, variants of lower MVA pathway polypeptides that confer the result of better isoprene production can also be used as well.

[0231] In some aspects, the lower MVA pathway polypeptide is a polypeptide from *Saccharomyces cerevisiae, Enterococcus faecalis,* or *Methanosarcina mazei.* In some aspects, the MVK polypeptide is selected from the group consisting of *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus* sakei mevalonate kinase polypeptide, yeast mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, *Streptomyces* mevalonate kinase polypeptide, *Streptomyces* CL190 mevalonate kinase polypeptide, *Methanosarcina mazei* mevalonate kinase polypeptide, and *M. Burtonii* mevalonate kinase polypeptide. Any one of the promoters described herein (*e.g.*, promoters described herein and identified in the Examples of the present disclosure including inducible promoters and constitutive promoters) can be used to drive expression of any of the MVA polypeptides described herein.

[0232] Any one of the cells described herein can comprise IDI nucleic acid(s) (*e.g.*, endogenous or heterologous nucleic acid(s) encoding IDI). Isopentenyl diphosphate isomerase polypeptides (isopentenyl-diphosphate delta-isomerase or IDI) catalyzes the interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (*e.g.*, converting IPP into DMAPP and/or converting DMAPP into IPP). Exemplary IDI polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of an IDI polypeptide. Standard methods (such as those described herein) can be used to determine whether a polypeptide has IDI polypeptide activity by measuring the ability of the polypeptide to interconvert IPP and DMAPP *in vitro*, in a cell extract, or *in vivo*. Exemplary IDI nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of an IDI polypeptide. Exemplary IDI polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein.

Nucleic acids encoding isoprene synthase polypeptides

[0233] In some aspects of the invention, the cells described in any of the compositions or methods described herein (including host cells that have been engineered for increased carbon flux toward the MVA pathway as described herein) further comprise one or more nucleic acids encoding an isoprene synthase polypeptide or a polypeptide having isoprene synthase activity.

In some aspects, the isoprene synthase polypeptide is an endogenous polypeptide. In some aspects, the endogenous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a constitutive promoter. In some aspects, the endogenous nucleic acid encoding an isoprene synthase polypeptide is operably linked to an inducible promoter. In some aspects, the endogenous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a strong promoter. In a particular aspect, the cells are engineered to over-express the endogenous isoprene synthase pathway polypeptide relative to wild-type cells. In some aspects, the endogenous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a weak promoter. In some aspects, the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid such as *Populus alba x Populus tremula.*

[0234] In some aspects, the isoprene synthase polypeptide is a heterologous polypeptide. In some aspects, the cells comprise more than one copy of a heterologous nucleic acid encoding an isoprene synthase polypeptide. In some aspects, the heterologous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a constitutive promoter. In some aspects, the heterologous nucleic acid encoding an isoprene synthase polypeptide is operably linked to an inducible promoter. In some aspects, the heterologous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a strong promoter. In some aspects, the heterologous nucleic acid encoding an isoprene synthase polypeptide is operably linked to a weak promoter.

[0235] The nucleic acids encoding an isoprene synthase polypeptide(s) can be integrated into a genome of the host cells or can be stably expressed in the cells. The nucleic acids encoding an isoprene synthase polypeptide(s) can additionally be on a vector.

[0236] Exemplary isoprene synthase nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of an isoprene synthase polypeptide. Isoprene synthase polypeptides convert dimethylallyl diphosphate (DMAPP) into isoprene. Exemplary isoprene synthase polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of an isoprene synthase polypeptide. Exemplary isoprene synthase polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein. In addition, variants of isoprene synthase can possess improved activity such as improved enzymatic activity. In some aspects, an isoprene

synthase variant has other improved properties, such as improved stability (*e.g.*, thermo-stability), and/or improved solubility.

[0237] Standard methods can be used to determine whether a polypeptide has isoprene synthase polypeptide activity by measuring the ability of the polypeptide to convert DMAPP into isoprene *in vitro*, in a cell extract, or *in vivo*. Isoprene synthase polypeptide activity in the cell extract can be measured, for example, as described in Silver *et al.*, *J. Biol. Chem.* 270:13010-13016, 1995. In one exemplary assay, DMAPP (Sigma) can be evaporated to dryness under a stream of nitrogen and rehydrated to a concentration of 100 mM in 100 mM potassium phosphate buffer pH 8.2 and stored at -20 °C. To perform the assay, a solution of 5 μL of 1M $MgCl_2$, 1 mM (250 μg/ml) DMAPP, 65 μL of Plant Extract Buffer (PEB) (50 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$, 5% glycerol, and 2 mM DTT) can be added to 25 μL of cell extract in a 20 ml Headspace vial with a metal screw cap and teflon coated silicon septum (Agilent Technologies) and cultured at 37 °C for 15 minutes with shaking. The reaction can be quenched by adding 200 μL of 250 mM EDTA and quantified by GC/MS.

[0238] In some aspects, the isoprene synthase polypeptide is a plant isoprene synthase polypeptide or a variant thereof. In some aspects, the isoprene synthase polypeptide is an isoprene synthase from *Pueraria* or a variant thereof. In some aspects, the isoprene synthase polypeptide is an isoprene synthase from Populus or a variant thereof. In some aspects, the isoprene synthase polypeptide is a poplar isoprene synthase polypeptide or a variant thereof. In some aspects, the isoprene synthase polypeptide is a kudzu isoprene synthase polypeptide or a variant thereof. In some aspects, the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula*, or a variant thereof.

[0239] In some aspects, the isoprene synthase polypeptide or nucleic acid is from the family Fabaceae, such as the Faboideae subfamily. In some aspects, the isoprene synthase polypeptide or nucleic acid is a polypeptide or nucleic acid from *Pueraria montana* (kudzu) (Sharkey *et al.*, *Plant Physiology* 137: 700-712, 2005), *Pueraria lobata*, poplar (such as *Populus alba*, *Populus nigra*, *Populus trichocarpa*, or *Populus alba x tremula* (CAC35696) (Miller *et al.*, *Planta* 213: 483-487, 2001), aspen (such as *Populus tremuloides*) (Silver *et al.*, JBC 270(22): 13010-1316, 1995), English Oak (*Quercus robur*) (Zimmer *et al.*, WO 98/02550), or a variant thereof. In some aspects, the isoprene synthase polypeptide is an isoprene synthase from

Pueraria montana, *Pueraria lobata*, *Populus tremuloides*, *Populus alba*, *Populus nigra*, or *Populus trichocarpa or* a variant thereof. In some aspects, the isoprene synthase polypeptide is an isoprene synthase from *Populus alba* or a variant thereof. In some aspects, the nucleic acid encoding the isoprene synthase (*e.g.*, isoprene synthase from *Populus alba* or a variant thereof) is codon optimized.

[0240] In some aspects, the isoprene synthase nucleic acid or polypeptide is a naturally-occurring polypeptide or nucleic acid (*e.g.*, naturally-occurring polypeptide or nucleic acid from *Populus*). In some aspects, the isoprene synthase nucleic acid or polypeptide is not a wild-type or naturally-occurring polypeptide or nucleic acid. In some aspects, the isoprene synthase nucleic acid or polypeptide is a variant of a wild-type or naturally-occurring polypeptide or nucleic acid (*e.g.*, a variant of a wild-type or naturally-occurring polypeptide or nucleic acid from *Populus*).

[0241] In some aspects, the isoprene synthase polypeptide is a variant. In some aspects, the isoprene synthase polypeptide is a variant of a wild-type or naturally occurring isoprene synthase. In some aspects, the variant has improved activity such as improved catalytic activity compared to the wild-type or naturally occurring isoprene synthase. The increase in activity (*e.g.*, catalytic activity) can be at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In some aspects, the increase in activity such as catalytic activity is at least about any of 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 30 folds, 40 folds, 50 folds, 75 folds, or 100 folds. In some aspects, the increase in activity such as catalytic activity is about 10% to about 100 folds (e.g., about 20% to about 100 folds, about 50% to about 50 folds, about 1 fold to about 25 folds, about 2 folds to about 20 folds, or about 5 folds to about 20 folds). In some aspects, the variant has improved solubility compared to the wild-type or naturally occurring isoprene synthase. The increase in solubility can be at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. The increase in solubility can be at least about any of 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 30 folds, 40 folds, 50 folds, 75 folds, or 100 folds. In some aspects, the increase in solubility is about 10% to about 100 folds (e.g., about 20% to about 100 folds, about 50% to about 50 folds, about 1 fold to about 25 folds, about 2 folds to about 20 folds, or about 5 folds to about 20 folds). In some aspects, the isoprene synthase polypeptide is a variant of naturally occurring isoprene synthase and has improved stability (such as thermo-stability) compared to the naturally occurring isoprene synthase.

[0242] In some aspects, the variant has at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, at least about 200% of the activity of a wild-type or naturally occurring isoprene synthase. The variant can share sequence similarity with a wild-type or naturally occurring isoprene synthase. In some aspects, a variant of a wild-type or naturally occurring isoprene synthase can have at least about any of 40%, 50%, 60%, 70%, 75%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% amino acid sequence identity as that of the wild-type or naturally occurring isoprene synthase. In some aspects, a variant of a wild-type or naturally occurring isoprene synthase has any of about 70% to about 99.9%, about 75% to about 99%, about 80% to about 98%, about 85% to about 97%, or about 90% to about 95% amino acid sequence identity as that of the wild-type or naturally occurring isoprene synthase.

[0243] In some aspects, the variant comprises a mutation in the wild-type or naturally occurring isoprene synthase. In some aspects, the variant has at least one amino acid substitution, at least one amino acid insertion, and/or at least one amino acid deletion. In some aspects, the variant has at least one amino acid substitution. In some aspects, the number of differing amino acid residues between the variant and wild-type or naturally occurring isoprene synthase can be one or more, *e.g.* 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more amino acid residues. Naturally occurring isoprene synthases can include any isoprene synthases from plants, for example, kudzu isoprene synthases, poplar isoprene synthases, English oak isoprene synthases, and willow isoprene synthases. In some aspects, the variant is a variant of isoprene synthase from *Populus alba*. In some aspects, the variant of isoprene synthase from *Populus alba* has at least one amino acid substitution, at least one amino acid insertion, and/or at least one amino acid deletion. In some aspects, the variant is a truncated *Populus alba* isoprene synthase. In some aspects, the nucleic acid encoding variant (*e.g.*, variant of isoprene synthase from *Populus alba*) is codon optimized (for example, codon optimized based on host cells where the heterologous isoprene synthase is expressed).

[0244] The isoprene synthase polypeptide provided herein can be any of the isoprene synthases or isoprene synthase variants described in WO 2009/132220, WO 2010/124146, and

U.S. Patent Application Publication No.: 2010/0086978, the contents of which are expressly incorporated herein by reference in their entirety with respect to the isoprene synthases and isoprene synthase variants.

[0245] Any one of the promoters described herein (*e.g.*, promoters described herein and identified in the Examples of the present disclosure including inducible promoters and constitutive promoters) can be used to drive expression of any of the isoprene synthases described herein.

[0246] Suitable isoprene synthases include, but are not limited to, those identified by Genbank Accession Nos. AY341431, AY316691, AY279379, AJ457070, and AY182241. Types of isoprene synthases which can be used in any one of the compositions or methods including methods of making cells encoding isoprene synthase described herein are also described in International Patent Application Publication Nos. WO2009/076676, WO2010/003007, WO2009/132220, WO2010/031062, WO2010/031068, WO2010/031076, WO2010/013077, WO2010/031079, WO2010/148150, WO2010/124146, WO2010/078457, WO2010/148256, WO 2012/058494, and US Patent No. 8,173,410.

Nucleic acids encoding DXP pathway polypeptides

[0247] In some aspects of the invention, the cells described in any of the compositions or methods described herein (including host cells that have been engineered for increased carbon flux toward the MVA pathway as described herein) further comprise one or more heterologous nucleic acids encoding a DXS polypeptide or other DXP pathway polypeptides. In some aspects, the cells further comprise a chromosomal copy of an endogenous nucleic acid encoding a DXS polypeptide or other DXP pathway polypeptides. In some aspects, the *E. coli* cells further comprise one or more nucleic acids encoding an IDI polypeptide and a DXS polypeptide or other DXP pathway polypeptides. In some aspects, one nucleic acid encodes the isoprene synthase polypeptide, IDI polypeptide, and DXS polypeptide or other DXP pathway polypeptides. In some aspects, one plasmid encodes the isoprene synthase polypeptide, IDI polypeptide, and DXS polypeptide or other DXP pathway polypeptides. In some aspects, multiple plasmids encode the isoprene synthase polypeptide, IDI polypeptide, and DXS polypeptide or other DXP pathway polypeptides.

[0248] Exemplary DXS polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of a DXS polypeptide. Standard methods (such as those described herein) can be used to determine whether a polypeptide has DXS polypeptide activity by measuring the ability of the polypeptide to convert pyruvate and D-glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose-5-phosphate *in vitro*, in a cell extract, or *in vivo*. Exemplary DXS polypeptides and nucleic acids and methods of measuring DXS activity are described in more detail in International Publication Nos. WO 2009/076676, WO 2010/003007, WO 2009/132220, and U.S. Patent Publ. Nos. US 2009/0203102, 2010/0003716 and 2010/0048964.

[0249] Exemplary DXP pathways polypeptides include, but are not limited to any of the following polypeptides: DXS polypeptides, DXR polypeptides, MCT polypeptides, CMK polypeptides, MCS polypeptides, HDS polypeptides, HDR polypeptides, and polypeptides (*e.g.*, fusion polypeptides) having an activity of one, two, or more of the DXP pathway polypeptides. In particular, DXP pathway polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of a DXP pathway polypeptide. Exemplary DXP pathway nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a DXP pathway polypeptide. Exemplary DXP pathway polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein as well as mutant polypeptides and nucleic acids derived from any of the source organisms described herein. Exemplary DXP pathway polypeptides and nucleic acids and methods of measuring DXP pathway polypeptide activity are described in more detail in International Publication No. WO 2010/148150

[0250] Exemplary DXS polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of a DXS polypeptide. Standard methods (such as those described herein) can be used to determine whether a polypeptide has DXS polypeptide activity by measuring the ability of the polypeptide to convert pyruvate and D-glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose-5-phosphate *in vitro*, in a cell extract, or *in vivo*. Exemplary DXS polypeptides and nucleic acids and methods of measuring DXS activity are described in more detail in International Publication No. WO 2009/076676, WO

2010/003007, WO 2009/132220, and U.S. Patent Publ. Nos. US 2009/0203102, 2010/0003716, and 2010/0048964.

[0251] In particular, DXS polypeptides convert pyruvate and D-glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose 5-phosphate (DXP). Standard methods can be used to determine whether a polypeptide has DXS polypeptide activity by measuring the ability of the polypeptide to convert pyruvate and D-glyceraldehyde 3-phosphate *in vitro*, in a cell extract, or *in vivo*.

[0252] DXR polypeptides convert 1-deoxy-D-xylulose 5-phosphate (DXP) into 2-C-methyl-D-erythritol 4-phosphate (MEP). Standard methods can be used to determine whether a polypeptide has DXR polypeptides activity by measuring the ability of the polypeptide to convert DXP *in vitro*, in a cell extract, or *in vivo*.

[0253] MCT polypeptides convert 2-C-methyl-D-erythritol 4-phosphate (MEP) into 4-(cytidine 5'-diphospho)-2-methyl-D-erythritol (CDP-ME). Standard methods can be used to determine whether a polypeptide has MCT polypeptides activity by measuring the ability of the polypeptide to convert MEP *in vitro*, in a cell extract, or *in vivo*.

[0254] CMK polypeptides convert 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-ME) into 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-MEP). Standard methods can be used to determine whether a polypeptide has CMK polypeptides activity by measuring the ability of the polypeptide to convert CDP-ME *in vitro*, in a cell extract, or *in vivo*.

[0255] MCS polypeptides convert 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-MEP) into 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate (ME-CPP or cMEPP). Standard methods can be used to determine whether a polypeptide has MCS polypeptides activity by measuring the ability of the polypeptide to convert CDP-MEP *in vitro*, in a cell extract, or *in vivo*.

[0256] HDS polypeptides convert 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate into (E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate (HMBPP or HDMAPP). Standard methods can be used to determine whether a polypeptide has HDS polypeptides activity by measuring the ability of the polypeptide to convert ME-CPP *in vitro*, in a cell extract, or *in vivo*.

[0257] HDR polypeptides convert (E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Standard methods can be used to determine whether a polypeptide has HDR polypeptides activity by measuring the ability of the polypeptide to convert HMBPP *in vitro*, in a cell extract, or *in vivo*.

Source organisms for lower MVA pathway, isoprene synthase, IDI, and DXP pathway polypeptides

[0258] Isoprene synthase, IDI, DXP pathway, and/or lower MVA pathway nucleic acids (and their encoded polypeptides) can be obtained from any organism that naturally contains isoprene synthase, IDI, DXP pathway, and/or lower MVA pathway nucleic acids. Isoprene is formed naturally by a variety of organisms, such as bacteria, yeast, plants, and animals. Some organisms contain the MVA pathway for producing isoprene. Isoprene synthase nucleic acids can be obtained, *e.g.*, from any organism that contains an isoprene synthase. MVA pathway nucleic acids can be obtained, *e.g.*, from any organism that contains the MVA pathway. IDI and DXP pathway nucleic acids can be obtained, *e.g.*, from any organism that contains the IDI and DXP pathway.

[0259] The nucleic acid sequence of the isoprene synthase, DXP pathway, IDI, and/or MVA pathway nucleic acids can be isolated from a bacterium, fungus, plant, algae, or cyanobacterium. Exemplary source organisms include, for example, yeasts, such as species of *Saccharomyces* (*e.g.*, *S. cerevisiae*), bacteria, such as species of *Escherichia* (*e.g.*, *E. coli*), or species of *Methanosarcina* (*e.g.*, *Methanosarcina mazei*), plants, such as kudzu or poplar (*e.g.*, *Populus alba* or *Populus alba x tremula* CAC35696) or aspen (*e.g.*, *Populus tremuloides*). Exemplary sources for isoprene synthases, IDI, and/or MVA pathway polypeptides which can be used are also described in International Patent Application Publication Nos. WO2009/076676, WO2010/003007, WO2009/132220, WO2010/031062, WO2010/031068, WO2010/031076, WO2010/013077, WO2010/031079, WO2010/148150, WO2010/078457, and WO2010/148256.

[0260] In some aspects, the source organism is a yeast, such as *Saccharomyces sp.*, *Schizosaccharomyces sp.*, *Pichia sp.*, or *Candida sp.*

[0261] In some aspects, the source organism is a bacterium, such as strains of *Bacillus* such as *B. lichenformis* or *B. subtilis*, strains of *Pantoea* such as *P. citrea*, strains of *Pseudomonas* such as *P. alcaligenes*, strains of *Streptomyces* such as *S. lividans* or *S.*

rubiginosus, strains of *Escherichia* such as *E. coli,* strains of *Enterobacter*, strains of *Streptococcus*, or strains of *Archaea* such as *Methanosarcina mazei.*

[0262] As used herein, "the genus *Bacillus*" includes all species within the genus "*Bacillus*," as known to those of skill in the art, including but not limited to *B. subtilis*, *B. licheniformis, B. lentus, B. brevis, B.* stearothermophilus, *B. alkalophilus, B. amyloliquefaciens*, *B. clausii*, *B. halodurans*, *B. megaterium, B. coagulans, B. circulans, B. lautus*, and *B. thuringiensis*. It is recognized that the genus *Bacillus* continues to undergo taxonomical reorganization. Thus, it is intended that the genus include species that have been reclassified, including but not limited to such organisms as *B.* stearothermophilus, which is now named "*Geobacillus stearothermophilus*." The production of resistant endospores in the presence of oxygen is considered the defining feature of the genus *Bacillus*, although this characteristic also applies to the recently named *Alicyclobacillus*, *Amphibacillus*, *Aneurinibacillus*, *Anoxybacillus*, *Brevibacillus*, *Filobacillus*, *Gracilibacillus*, *Halobacillus*, *Paenibacillus*, *Salibacillus*, *Thermobacillus*, *Ureibacillus*, and *Virgibacillus*.

[0263] In some aspects, the source organism is a gram-positive bacterium. Non-limiting examples include strains of *Streptomyces* (*e.g.*, *S. lividans*, *S. coelicolor*, or *S. griseus*) and *Bacillus*. In some aspects, the source organism is a gram–negative bacterium, such as *E. coli* or *Pseudomonas sp.*

[0264] In some aspects, the source organism is a plant, such as a plant from the family Fabaceae, such as the Faboideae subfamily. In some aspects, the source organism is kudzu, poplar (such as *Populus alba x tremula* CAC35696), aspen (such as *Populus tremuloides*), or *Quercus robur.*

[0265] In some aspects, the source organism is an algae, such as a green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, or dinoflagellates.

[0266] In some aspects, the source organism is a cyanobacteria, such as cyanobacteria classified into any of the following groups based on morphology: *Chroococcales*, *Pleurocapsales*, *Oscillatoriales*, *Nostocales*, or *Stigonematales.*

Recombinant cells capable of increased production of isoprene

[0267] The recombinant cells described herein (including host cells that have been engineered for increased carbon flux as described herein) have the ability to produce isoprene concentration greater than that of the same cells lacking one or more copies of a heterologous nucleic acid phosphoketolase polypeptides, one or more copies of a heterologous nucleic acid encoding a MVA pathway polypeptide, and one or more heterologous nucleic acids encoding an isoprene synthase polypeptide when cultured under the same conditions. The cells can further comprise one or more heterologous nucleic acids encoding an IDI polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment,

the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0268] In some aspects, the one or more copies of a heterologous nucleic acid encoding phosphoketolase, one or more copies of a heterologous nucleic acid encoding a MVA pathway polypeptide, and one or more heterologous nucleic acids encoding an isoprene synthase polypeptide are heterologous nucleic acids that are integrated into the host cell's chromosomal nucleotide sequence. In other aspects, the one or more heterologous nucleic acids are integrated into plasmid. In still other aspects, at least one of the one or more heterologous nucleic acids is integrated into the cell's chromosomal nucleotide sequence while at least one of the one or more heterologous nucleic acid sequences is integrated into a plasmid. The recombinant cells can produce at least 5% greater amounts of isoprene compared to isoprene-producing cells that do not comprise the phosphoketolase polypeptide. Alternatively, the recombinant cells can produce greater than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% of isoprene, inclusive, as well as any numerical value in between these numbers.

[0269] In one aspect of the invention, provided herein are recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, one or more heterologous nucleic acids encoding a mevalonate (MVA) pathway polypeptide(s), one or more heterologous nucleic acids encoding a DXP pathway polypeptide(s), and one or more heterologous nucleic acids encoding an isoprene synthase polypeptide. The cells can further comprise one or more heterologous nucleic acids encoding an IDI polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid

encoding a phosphoketolase isolated from *Ferrimonas balearica*. In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*. In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*. Any of the one or more heterologous nucleic acids can be operably linked to constitutive promoters, can be operably linked to inducible promoters, or can be operably linked to a combination of inducible and constitutive promoters. The one or more heterologous nucleic acids can additionally be operably linked to strong promoters, weak promoters, and/or medium promoters. One or more of the heterologous nucleic acids encoding phosphoketolase, a mevalonate (MVA) pathway polypeptide(s), a DXP pathway polypeptide(s), and an isoprene synthase polypeptide can be integrated into a genome of the host cells or can be stably expressed in the cells. The one or more heterologous nucleic acids can additionally be on a vector.

[0270] The production of isoprene by the cells according to any of the compositions or methods described herein can be enhanced (*e.g.*, enhanced by the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, an isoprene synthase polypeptide, MVA pathway polypeptide(s), and/or a DXP pathway polypeptide(s)). As used herein, "enhanced" isoprene production refers to an increased cell productivity index (CPI) for isoprene, an increased titer of isoprene, an increased mass yield of isoprene, and/or an increased specific productivity of isoprene by the cells described by any of the compositions and methods described herein compared to cells which do not have one or more heterologous nucleic acids encoding a phosphoketolase peptide. In certain embodiments described herein, the host cells have been further engineered increased carbon flux to MVA production.

[0271] The production of isoprene by the recombinant cells described herein can be enhanced by about 5% to about 1,000,000 folds. In certain aspects, the production of isoprene can be enhanced by about 10% to about 1,000,000 folds (*e.g.*, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprene by cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide. In certain embodiments described herein, the host cells have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprene as compared to the production of isoprene by cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production.

[0272] In other aspects, the production of isoprene by the recombinant cells described herein can also be enhanced by at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 50 folds, 100 folds, 200 folds, 500 folds, 1000 folds, 2000 folds, 5000 folds, 10,000 folds, 20,000 folds, 50,000 folds, 100,000 folds, 200,000 folds, 500,000 folds, or 1,000,000 folds as compared to the production of isoprene by cells that do not express one or more heterologous nucleic acids encoding

phosphoketolase peptide. In certain embodiments described herein, the host cells have been further engineered increased carbon flux to MVA production thereby providing enhanced production of isoprene as compared to the production of isoprene by cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production.

Methods of using the recombinant cells to produce isoprene

[0273] Also provided herein are methods for producing isoprene comprising culturing any of the recombinant cells described herein. In one aspect, isoprene can be produced by culturing recombinant cells comprising one or more heterologous nucleic acids encoding an phosphoketolase polypeptide, one or more MVA pathway polypeptides, and an isoprene synthase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis*

dassonvillei, and/or Thermobifida fusca. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0274] In another aspect, isoprene can be produced by culturing recombinant cells comprising modulation in any of the enzymatic pathways described herein and one or more heterologous nucleic acids encoding a phosphoketolase peptide, a MVA pathway polypeptide, and an isoprene synthase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* The isoprene can be produced from any of the cells described herein and according to any of the methods described herein. Any of

the cells can be used for the purpose of producing isoprene from carbohydrates, including, but not limited to, six carbon sugars such as glucose. In other embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0275] Thus, provided herein are methods of producing isoprene comprising culturing cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide and an isoprene synthase in a suitable condition for producing isoprene and (b) producing isoprene. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis,*

Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0276] The cells can further comprise one or more nucleic acid molecules encoding the MVA pathway polypeptide(s) described above (e.g., the complete MVA pathway) and any of the isoprene synthase polypeptide(s) described above (e.g. *Pueraria* isoprene synthase). In some aspects, the recombinant cells can be one of any of the cells described herein. Any of the isoprene synthases or variants thereof described herein, any of the host cell strains described herein, any of the promoters described herein, and/or any of the vectors described herein can also be used to produce isoprene using any of the energy sources (e.g. glucose or any other six carbon sugar) described herein can be used in the methods described herein. In some aspects, the method of producing isoprene further comprises a step of recovering the isoprene. In other embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.*

[0277] In certain aspects, provided herein are methods of making isoprene comprising culturing recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum,*

Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca, an mvaE and an mvaS polypeptide from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis*, in a suitable condition for producing isoprene and (b) producing isoprene. The cells can further comprise one or more nucleic acid molecules encoding the lower MVA pathway polypeptide(s) described above (e.g., MVK, PMK, MVD, and/or IDI) and any of the isoprene synthase polypeptide(s) described above. In some aspects, the recombinant cells can be any of the cells described herein.

[0278] In certain aspects, provided herein are methods of making isoprene comprising culturing recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca,* in a suitable condition for producing isoprene and (b) producing isoprene. The cells can further comprise one or more nucleic acid molecules encoding the lower MVA pathway polypeptide(s) described above (e.g., MVK, PMK, MVD, and/or IDI) and any of the isoprene synthase polypeptide(s) described above. In some aspects, the recombinant cells can be any of the cells described herein. The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flow to mevalonate production can be used to produce isoprene. In some aspects, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of rribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphate acetyltransferase (*pta* and/or *eutD*). In another embodiment, these recombinant cells can be further engineered to decrease the activity of one or more genes of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA*

and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*).

[0279] In certain aspects, provided herein are methods of making isoprene comprising culturing recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum,* in a suitable condition for producing isoprene and (b) producing isoprene. The cells can further comprise one or more nucleic acid molecules encoding the lower MVA pathway polypeptide(s) described above (e.g., MVK, PMK, MVD, and/or IDI) and any of the isoprene synthase polypeptide(s) described above. In some aspects, the recombinant cells can be any of the cells described herein. The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flow to mevalonate production can be used to produce isoprene. In some aspects, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of rpiA, rpe, tktA, tal B, pta and/or eutD. In another aspect, these strains can be further engineered to decrease the activity of one or more genes of the following genes including zwf, pfkA, fba, gapA, ackA, gltA and/or pts.

[0280] In some aspects, the amount of isoprene produced is measured at the peak absolute productivity time point. In some aspects, the peak absolute productivity for the cells is about any of the amounts of isoprene disclosed herein. In some aspects, the amount of isoprene produced is measured at the peak specific productivity time point. In some aspects, the peak specific productivity for the cells is about any of the amounts of isoprene per cell disclosed herein. In some aspects, the cumulative, total amount of isoprene produced is measured. In some aspects, the cumulative total productivity for the cells is about any of the amounts of isoprene disclosed herein.

[0281] In some aspects, any of the cells described herein (for examples the cells in culture) produce isoprene at greater than about any of or about any of 1, 10, 25, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,500, 1,750, 2,000, 2,500, 3,000, 4,000, 5,000, or more nmole of isoprene/gram of cells for the wet weight of the cells/hour (nmole/g_{wcm}/hr). In some aspects, the amount of isoprene is between about 2 to about 5,000 nmole/g_{wcm}/hr, such as between about 2 to about 100 nmole/g_{wcm}/hr, about 100 to about 500 nmole/g_{wcm}/hr, about 150 to about 500 nmole/g_{wcm} /hr, about 500 to about 1,000 nmole/g_{wcm}/hr,

about 1,000 to about 2,000 nmole/g_{wcm}/hr, or about 2,000 to about 5,000 nmole/g_{wcm}/hr. In some aspects, the amount of isoprene is between about 20 to about 5,000 nmole/g_{wcm}/hr, about 100 to about 5,000 nmole/g_{wcm}/hr, about 200 to about 2,000 nmole/g_{wcm}/hr, about 200 to about 1,000 nmole/g_{wcm}/hr, about 300 to about 1,000 nmole/g_{wcm}/hr, or about 400 to about 1,000 nmole/g_{wcm}/hr.

[0282] In some aspects, the cells in culture produce isoprene at greater than or about 1, 10, 25, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,500, 1,750, 2,000, 2,500, 3,000, 4,000, 5,000, 10,000, 100,000, or more ng of isoprene/gram of cells for the wet weight of the cells/hr (ng/g_{wcm}/h). In some aspects, the amount of isoprene is between about 2 to about 5,000 ng/g_{wcm}/h, such as between about 2 to about 100 ng/g_{wcm}/h, about 100 to about 500 ng/g_{wcm}/h, about 500 to about 1,000 ng/g_{wcm}/h, about 1,000 to about 2,000 ng/g_{wcm}/h, or about 2,000 to about 5,000 ng/g_{wcm}/h. In some aspects, the amount of isoprene is between about 20 to about 5,000 ng/g_{wcm}/h, about 100 to about 5,000 ng/g_{wcm}/h, about 200 to about 2,000 ng/g_{wcm}/h, about 200 to about 1,000 ng/g_{wcm}/h, about 300 to about 1,000 ng/g_{wcm}/h, or about 400 to about 1,000 ng/g_{wcm}/h.

[0283] In some aspects, the cells in culture produce a cumulative titer (total amount) of isoprene at greater than about any of or about any of 1, 10, 25, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,500, 1,750, 2,000, 2,500, 3,000, 4,000, 5,000, 10,000, 50,000, 100,000, or more mg of isoprene/L of broth (mg/L_{broth}, wherein the volume of broth includes the volume of the cells and the cell medium). In some aspects, the amount of isoprene is between about 2 to about 5,000 mg/L_{broth}, such as between about 2 to about 100 mg/L_{broth}, about 100 to about 500 mg/L_{broth}, about 500 to about 1,000 mg/L_{broth}, about 1,000 to about 2,000 mg/L_{broth}, or about 2,000 to about 5,000 mg/L_{broth}. In some aspects, the amount of isoprene is between about 20 to about 5,000 mg/L_{broth}, about 100 to about 5,000 mg/L_{broth}, about 200 to about 2,000 mg/L_{broth}, about 200 to about 1,000 mg/L_{broth}, about 300 to about 1,000 mg/L_{broth}, or about 400 to about 1,000 mg/L_{broth}.

[0284] In some aspects, the isoprene produced by the cells in culture comprises at least about 1, 2, 5, 10, 15, 20, or 25% by volume of the fermentation offgas. In some aspects, the isoprene comprises between about 1 to about 25% by volume of the offgas, such as between about 5 to about 15 %, about 15 to about 25%, about 10 to about 20%, or about 1 to about 10 %.

[0285] In certain embodiments, the methods of producing isoprene can comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously express a phosphoketolase polypeptide, wherein the cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide along with (i) one or more nucleic acids expressing one or more MVA pathway peptides and (ii) an isoprene synthase and (b) producing isoprene, wherein the recombinant cells display decreased oxygen uptake rate (OUR) as compared to that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide. In certain embodiments, the recombinant cells expressing one or more heterologous copies of a gene encoding an phosphoketolase polypeptide display up to 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold or 7-fold decrease in OUR as compared to recombinant cells that do not express a phosphoketolase.

[0286] Also provided herein are methods the the production of isoprene comprising cells having enhanced isoprene production capabilities. The production of isoprene by the cells described herein can be enhanced by the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, one or more copies of a heterologous nucleic acid encoding one or more polypeptides of the complete MVA pathway polypeptide, and one or more heterologous nucleic acids encoding an isoprene synthase polypeptide. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase

isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* As used herein, "enhanced" isoprene production refers to an increased cell productivity index (CPI) for isoprene, an increased titer of isoprene, an increased mass yield of isoprene, and/or an increased specific productivity of isoprene by the cells described by any of the compositions and methods described herein compared to cells which do not have one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, a MVA pathway polypeptide(s) and an isoprene synthase polypeptide. The production of isoprene can be enhanced by about 5% to about 1,000,000 folds. The production of isoprene can be enhanced by about 10% to about 1,000,000 folds (*e.g.*, about 50% to about 1,000,000 folds, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprene by the isoprene-producing cells that do not endogenously express phosphoketolase enzyme. In certain embodiments described herein, the methods described herein comprise host cells have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprene as compared to the

production of isoprene by isoprene-producing cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production. In certain embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.*

[0287] In other aspects, the methods described herein are directed to the enhanced production of isoprene by the cells described herein (*e.g.*, enhanced by the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide). In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment,

the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* The production of isoprene can be enhanced by about 5% to about 1,000,000 folds. The production of isoprene can be enhanced by about 10% to about 1,000,000 folds (*e.g.*, about 50% to about 1,000,000 folds, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprene by an isoprene-producing cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. The production of isoprene can also enhanced by at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 50 folds, 100 folds, 200 folds, 500 folds, 1000 folds, 2000 folds, 5000 folds, 10,000 folds, 20,000 folds, 50,000 folds, 100,000 folds, 200,000 folds, 500,000 folds, or 1,000,000 folds compared to the production of isoprene by isoprene-producing cells without the expression of one or more heterologous nucleic acids encoding phosphoketolase. In certain embodiments described herein, the methods described herein comprise host cells have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprene as compared to the production of isoprene by cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production. In certain embodiments, the phosphoketolase polypeptide is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.*

[0288] In addition, more specific cell culture conditions can be used to culture the cells in the methods described herein. For example, in some aspects, the method for the production of isoprene comprises the steps of (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously have a phosphoketolase gene in minimal medium at 34°C,

wherein the recombinant cells heterologously express (i) one or more copies of a heterologous gene encoding a phosphoketolase polypeptide on a low to medium copy plasmid and under the control of a strong promoter, (ii) one or more copies of a heterologous nucleic acid encoding one or more polypeptides of the MVA pathway polypeptide (upper MVA pathway and lower MVA pathway), and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide; and (b) producing isoprene. In certain embodiments, the phosphoketolase polypeptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the

recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* In some aspects, the method of producing isoprene further comprises a step of recovering the isoprene.

Recombinant cells capable of increased production of isoprenoid precursors and/or isoprenoids

[0289] Isoprenoids can be produced in many organisms from the synthesis of the isoprenoid precursor molecules which are the end products of the MVA pathway. As stated above, isoprenoids represent an important class of compounds and include, for example, food and feed supplements, flavor and odor compounds, and anticancer, antimalarial, antifungal, and antibacterial compounds.

[0290] As a class of molecules, isoprenoids are classified based on the number of isoprene units comprised in the compound. Monoterpenes comprise ten carbons or two isoprene units, sesquiterpenes comprise 15 carbons or three isoprene units, diterpenes comprise 20 carbons or four isoprene units, sesterterpenes comprise 25 carbons or five isoprene units, and so forth. Steroids (generally comprising about 27 carbons) are the products of cleaved or rearranged isoprenoids.

[0291] Isoprenoids can be produced from the isoprenoid precursor molecules IPP and DMAPP. These diverse compounds are derived from these rather simple universal precursors and are synthesized by groups of conserved polyprenyl pyrophosphate synthases (Hsieh et al., *Plant Physiol.* 2011 Mar;155(3):1079-90). The various chain lengths of these linear prenyl pyrophosphates, reflecting their distinctive physiological functions, in general are determined by the highly developed active sites of polyprenyl pyrophosphate synthases via condensation reactions of allylic substrates (dimethylallyl diphosphate (C_5-DMAPP), geranyl pyrophosphate (C_{10}-GPP), farnesyl pyrophosphate (C_{15}-FPP), geranylgeranyl pyrophosphate (C_{20}-GGPP)) with corresponding number of isopentenyl pyrophosphates (C_5-IPP) (Hsieh et al., *Plant Physiol.* 2011 Mar;155(3):1079-90).

[0292] Production of isoprenoid precursors and/or isoprenoids can be made by using any of the recombinant host cells that comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase for increased production of isoprenoid precursors and/or isoprenoids. In some aspects, these cells further comprise one or more heterologous nucleic acids encoding polypeptides of the MVA pathway, IDI, and/or the DXP pathway, as described above, and a heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide. Without being bound to theory, it is thought that increasing the cellular production of mevalonate in recombinant cells by any of the compositions and methods described above will similarly result in the production of higher amounts of isoprenoid precursor molecules and/or isoprenoids. Increasing the molar yield of mevalonate production from glucose translates into higher molar yields of isoprenoid precursor molecules and/or isoprenoids, including isoprene, produced from glucose when combined with appropriate enzymatic activity levels of mevalonate kinase, phosphomevalonate kinase, diphosphomevalonate decarboxylase, isopentenyl diphosphate isomerase and other appropriate enzymes for isoprene and isoprenoid production. The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flow to mevalonate production can be used to produce isoprenoid precursors and/or isoprenoids. In some aspects, the recombinant cells can be further engineered to incease the activity of one or more of the following genes selected from the group consisting of rpiA, rpe, tktA, tal B, pta and/or eutD. In another aspect, these strains can be further engineered to decrease the activity of one or more genes of the following genes including zwf, pfkA, fba, gapA, ackA, gltA and/or pts.

Types of isoprenoids

[0293] The recombinant cells of the present invention are capable of increased production of isoprenoids and the isoprenoid precursor molecules DMAPP and IPP. Examples of isoprenoids include, without limitation, hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids, and higher polyterpenoids. In some aspects, the hemiterpenoid is prenol (*i.e.*, 3-methyl-2-buten-1-ol), isoprenol (*i.e.*, 3-methyl-3-buten-1-ol), 2-methyl-3-buten-2-ol, or isovaleric acid. In some aspects, the monoterpenoid can be, without limitation, geranyl pyrophosphate, eucalyptol, limonene, or pinene. In some aspects, the sesquiterpenoid is farnesyl pyrophosphate, artemisinin, or bisabolol. In some aspects, the diterpenoid can be, without limitation, geranylgeranyl pyrophosphate, retinol, retinal, phytol,

taxol, forskolin, or aphidicolin. In some aspects, the triterpenoid can be, without limitation, squalene or lanosterol. The isoprenoid can also be selected from the group consisting of abietadiene, amorphadiene, carene, α-famesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

[0294] In some aspects, the tetraterpenoid is lycopene or carotene (a carotenoid). As used herein, the term "carotenoid" refers to a group of naturally-occurring organic pigments produced in the chloroplasts and chromoplasts of plants, of some other photosynthetic organisms, such as algae, in some types of fungus, and in some bacteria. Carotenoids include the oxygen-containing xanthophylls and the non-oxygen-containing carotenes. In some aspects, the carotenoids are selected from the group consisting of xanthophylls and carotenes. In some aspects, the xanthophyll is lutein or zeaxanthin. In some aspects, the carotenoid is α-carotene, β-carotene, γ-carotene, β-cryptoxanthin or lycopene.

Heterologous nucleic acids encoding polyprenyl pyrophosphate synthases polypeptides

[0295] In some aspects of the invention, the cells described in any of the compositions or methods herein further comprise one or more nucleic acids encoding a mevalonate (MVA) pathway polypeptide(s), as described above, as well as one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptides(s). The polyprenyl pyrophosphate synthase polypeptide can be an endogenous polypeptide. The endogenous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide can be operably linked to a constitutive promoter or can similarly be operably linked to an inducible promoter. The endogenous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide can additionally be operably linked to a strong promoter. Alternatively, the endogenous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide can be operably linked to a weak promoter. In particular, the cells can be engineered to over-express the endogenous polyprenyl pyrophosphate synthase polypeptide relative to wild-type cells.

[0296] In some aspects, the polyprenyl pyrophosphate synthase polypeptide is a heterologous polypeptide. The cells of the present invention can comprise more than one copy of a heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide. In some aspects, the heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase

polypeptide is operably linked to a constitutive promoter. In some aspects, the heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide is operably linked to an inducible promoter. In some aspects, the heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide is operably linked to a strong promoter. In some aspects, the heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide is operably linked to a weak promoter.

[0297] The nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide(s) can be integrated into a genome of the host cells or can be stably expressed in the cells. The nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide(s) can additionally be on a vector.

[0298] Exemplary polyprenyl pyrophosphate synthase nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a polyprenyl pyrophosphate synthase. Polyprenyl pyrophosphate synthase polypeptides convert isoprenoid precursor molecules into more complex isoprenoid compounds. Exemplary polyprenyl pyrophosphate synthase polypeptides include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of an isoprene synthase polypeptide. Exemplary polyprenyl pyrophosphate synthase polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein. In addition, variants of polyprenyl pyrophosphate synthase can possess improved activity such as improved enzymatic activity. In some aspects, a polyprenyl pyrophosphate synthase variant has other improved properties, such as improved stability (*e.g.*, thermo-stability), and/or improved solubility. Exemplary polyprenyl pyrophosphate synthase nucleic acids can include nucleic acids which encode polyprenyl pyrophosphate synthase polypeptides such as, without limitation, geranyl diphosposphate (GPP) synthase, farnesyl pyrophosphate (FPP) synthase, and geranylgeranyl pyrophosphate (GGPP) synthase, or any other known polyprenyl pyrophosphate synthase polypeptide.

[0299] In some aspects of the invention, the cells described in any of the compositions or methods herein further comprise one or more nucleic acids encoding a farnesyl pyrophosphate (FPP) synthase. The FPP synthase polypeptide can be an endogenous polypeptide encoded by an endogenous gene. In some aspects, the FPP synthase polypeptide is encoded by an endogenous ispA gene in *E. coli*. The endogenous nucleic acid encoding an FPP synthase

polypeptide can be operably linked to a constitutive promoter or can similarly be operably linked to an inducible promoter. The endogenous nucleic acid encoding an FPP synthase polypeptide can additionally be operably linked to a strong promoter. In particular, the cells can be engineered to over-express the endogenous FPP synthase polypeptide relative to wild-type cells.

[0300] In some aspects, the FPP synthase polypeptide is a heterologous polypeptide. The cells of the present invention can comprise more than one copy of a heterologous nucleic acid encoding a FPP synthase polypeptide. In some aspects, the heterologous nucleic acid encoding a FPP synthase polypeptide is operably linked to a constitutive promoter. In some aspects, the heterologous nucleic acid encoding a FPP synthase polypeptide is operably linked to an inducible promoter. In some aspects, the heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide is operably linked to a strong promoter.

[0301] The nucleic acids encoding an FPP synthase polypeptide can be integrated into a genome of the host cells or can be stably expressed in the cells. The nucleic acids encoding an FPP synthase can additionally be on a vector.

[0302] Standard methods can be used to determine whether a polypeptide has polyprenyl pyrophosphate synthase polypeptide activity by measuring the ability of the polypeptide to convert IPP into higher order isoprenoids *in vitro*, in a cell extract, or *in vivo*. These methods are well known in the art and are described, for example, in U.S. Patent No.: 7,915,026; Hsieh et al., *Plant Physiol.* 2011 Mar;155(3):1079-90; Danner et al., *Phytochemistry.* 2011 Apr 12 [Epub ahead of print]; Jones et al., *J Biol Chem.* 2011 Mar 24 [Epub ahead of print]; Keeling et al., *BMC Plant Biol.* 2011 Mar 7;11:43; Martin et al., *BMC Plant Biol.* 2010 Oct 21;10:226; Kumeta & Ito, *Plant Physiol.* 2010 Dec;154(4):1998-2007; and Köllner & Boland, *J Org Chem.* 2010 Aug 20;75(16):5590-600.

Recombinant cells capable of increased production of isoprenoid precursors and/or isoprenoids

[0303] The recombinant cells (e.g., recombinant bacterial cells) described herein have the ability to produce isoprenoid precursors and/or isoprenoids at a amount and/or concentration greater than that of the same cells lacking one or more copies of a heterologous nucleic acid encoding phosphoketolase, one or more copies of a heterologous nucleic acid encoding a MVA pathway polypeptide, and one or more heterologous nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide when cultured under the same conditions. In certain

embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei.* In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* In some aspects, the one or more copies of a heterologous nucleic acid encoding phosphoketolase, one or more copies of a

heterologous nucleic acid encoding a MVA pathway polypeptide, and one or more heterologous nucleic acid encoding a polyprenyl pyrophosphate synthase polypeptide are heterologous nucleic acids that are integrated into the host cell's chromosome. The recombinant cells can produce at least 5% greater amounts of isoprenoid precursors and/or isoprenoids when compared to isoprenoids and/or isoprenoid precursor -producing recombinant cells that do not comprise phosphoketolase polypeptide. Alternatively, the recombinant cells can produce greater than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% of isoprenoid precursors and/or isoprenoids, inclusive, as well as any numerical value in between these numbers compared to the production of isoprenoids and/or isoprenoid-precursors by isoprenoids and/or isoprenoid-precursors- producing cells which do not express of one or more heterologous nucleic acids encoding a phosphoketolase. In certain embodiments described herein, the methods herein comprise host cells have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprenoids and/or isoprenoid-precursors as compared to the production of isoprenoids and/or isoprenoid-precursors by isoprenoids and/or isoprenoid-precursors-producing cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production.

[0304] In one aspect of the invention, there are provided recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, one or more heterologous nucleic acids encoding one or more complete MVA pathway polypeptide(s) (i.e., the upper MVA pathway and the lower MVA pathway), one or more heterologous nucleic acids encoding polyprenyl pyrophosphate synthase and/or one or more heterologous nucleic acids encoding a DXP pathway polypeptide(s). The cells can further comprise one or more heterologous nucleic acids encoding an IDI polypeptide. Additionally, the polyprenyl pyrophosphate synthase polypeptide can be an FPP synthase polypeptide. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the

recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica*. In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*. In other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*. The one or more heterologous nucleic acids can be operably linked to constitutive promoters, can be operably linked to inducible promoters, or can be operably linked to a combination of inducible and constitutive promoters. The one or more heterologous nucleic acids can additionally be operably linked strong promoters, weak promoters, and/or medium promoters. One or more of the heterologous nucleic acids encoding a phosphoketolase polypeptide, one or more complete MVA pathway polypeptide(s) (i.e., the upper MVA pathway and the lower MVA pathway) , a polyprenyl pyrophosphate synthase polypeptide and/or one or more heterologous nucleic acids encoding a DXP pathway polypeptide(s) can be integrated into a genome of the host cells or can be stably

expressed in the cells. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum.* The one or more heterologous nucleic acids can additionally be on one or more vectors.

[0305] Provided herein are recombinant cells which can provide enhanced isoprenoid precursor and/or isoprenoid production. The production of isoprenoid precursors and/or isoprenoids by the cells can be enhanced by the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, one or more heterologous nucleic acids encoding one or more polypeptide(s) of the complete MVA pathway (i.e., the upper MVA pathway and lower MVA pathway), and one or more heterologous nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide. In certain embodiments, the one or more copies of a heterologous nucleic acid encoding phosphoketolase peptide is from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus reuteri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Ferrimonas balearica.* In

yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Pedobactor saltans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptomyces griseus*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Nocardiopsis dassonvillei*. n other embodiments, the recombinant cells described herein comprise one ore more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium longum*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus gallinarum*. As used herein, "enhanced" isoprenoid precursor and/or isoprenoid production refers to an increased cell productivity index (CPI) for isoprenoid precursor and/or isoprenoid production, an increased titer of isoprenoid precursors and/or isoprenoids, an increased mass yield of isoprenoid precursors and/or isoprenoids, and/or an increased specific productivity of isoprenoid precursors and/or isoprenoids by the cells described by any of the compositions and methods described herein compared to cells which do not have one or more heterologous nucleic acids encoding a phosphoketolase, one or more polypeptide(s) of the complete MVA pathway, and a polyprenyl pyrophosphate synthase polypeptide. The production of isoprenoid precursors and/or isoprenoids can be enhanced by about 5% to about 1,000,000 folds. The production of isoprenoid precursors and/or isoprenoids can be enhanced by about 10% to about 1,000,000

folds (*e.g.*, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprenoid and/or isoprenoid precursors by cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase. In certain embodiments described herein, the recombinant host cells have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprenoids and/or isoprenoid-precursors as compared to the production of isoprenoids and/or isoprenoid-precursors by isoprenoids and/or isoprenoid-precursors-producing cells that do not express one or more heterologous nucleic acids encoding phosphoketolase polypeptide and which have not been engineered for increased carbon flux to mevalonate production.

[0306] The production of isoprenoid precursors and/or isoprenoids by the cells described herein can be enhanced (*e.g.*, enhanced by the expression of one or more heterologous nucleic acids encoding the phosphoketolase polypeptides from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca*, one or more heterologous nucleic acids encoding a lower MVA pathway polypeptide, and one or more heterologous nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide). The production of isoprenoid precursors and/or isoprenoids can be enhanced by about 5% to about 1,000,000 folds. The production of isoprenoid precursors and/or isoprenoids can be enhanced by about 10% to about 1,000,000 folds (*e.g.*, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about

1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprenoid precursors and/or isoprenoids by naturally-occurring cells (*e.g.*, cells without the expression of one or more heterologous nucleic acids encoding phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides and which have not been engineered for increased carbon flux to mevalonate production.

[0307] In other embodiments, the recombinant cells described herein can provide for the production of isoprenoid precursors and/or isoprenoids can also enhanced by at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 50 folds, 100 folds, 200 folds, 500 folds, 1000 folds, 2000 folds, 5000 folds, 10,000 folds, 20,000 folds, 50,000 folds, 100,000 folds, 200,000 folds, 500,000 folds, or 1,000,000 folds compared to the production of isoprenoid precursors and/or isoprenoids by isoprenoid precursors and/or isoprenoids producing recombinant cells which do not express of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide

Methods of using the recombinant cells to produce isoprenoids and/or isoprenoid precursor molecules

[0308] Also provided herein are methods of producing isoprenoid precursor molecules and/or isoprenoids comprising culturing recombinant cells (e.g., recombinant bacterial cells) that comprise one or more heterologous nucleic acids encoding a phosphoketolase and an polyprenyl pyrophosphate synthase polypeptide. In certain embodiments, the recombinant cells further comprise one or more one or more heterologous nucleic acids encoding an upper MVA pathway polypeptide and a lower MVA pathway polypeptide. The isoprenoid precursor molecules and/or isoprenoids can be produced from any of the cells described herein and according to any of the methods described herein. Any of the cells can be used for the purpose of producing isoprenoid precursor molecules and/or isoprenoids from carbohydrates, including six carbon sugars such as glucose.

[0309] In certain aspects, provided herein are methods of making isoprenoid precursor molecules and/or isoprenoids comprising culturing recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei*, an mvaE and an mvaS polypeptide from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis,* in a suitable condition for producing isoprenoid precursor molecules and/or isoprenoids, and (b) producing isoprenoid precursor molecules and/or isoprenoids. The cells can further comprise one or more nucleic acid molecules encoding the lower MVA pathway polypeptide(s) described above (e.g., MVK, PMK, MVD, and/or IDI) and any of the polyprenyl pyrophosphate synthase polypeptide(s) described above. In some aspects, the recombinant cells can be any of the cells described herein. Any of the polyprenyl pyrophosphate synthase or variants thereof described herein, any of the host cell strains described herein, any of the promoters described herein, and/or any of the vectors described herein can also be used to produce isoprenoid precursor molecules and/or isoprenoids using any of the energy sources (e.g. glucose or any other six carbon sugar) described herein. In some aspects, the method of producing isoprenoid precursor molecules and/or isoprenoids further comprises a step of recovering the isoprenoid precursor molecules and/or isoprenoids.

[0310] In certain aspects, provided herein are methods of making isoprenoid precursor molecules and/or isoprenoids comprising culturing recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum,* an mvaE and an mvaS polypeptide from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis,* in a suitable condition for producing isoprenoid precursor molecules and/or isoprenoids, and (b) producing isoprenoid precursor molecules and/or isoprenoids. The cells can further comprise one or more nucleic acid molecules encoding the lower MVA pathway polypeptide(s) described above (e.g., MVK, PMK, MVD, and/or IDI) and any of the polyprenyl pyrophosphate synthase polypeptide(s) described above. In some aspects, the recombinant cells can be any of the cells described herein. Any of the polyprenyl pyrophosphate synthase or variants thereof described herein, any of the host cell strains described herein, any of the promoters described herein, and/or any of the vectors described herein can also be used to produce isoprenoid precursor molecules and/or isoprenoids using any of the energy sources (e.g. glucose or any other six carbon sugar) described herein. In some aspects, the method of producing isoprenoid precursor

molecules and/or isoprenoids further comprises a step of recovering the isoprenoid precursor molecules and/or isoprenoids.

[0311] The method of producing isoprenoid precursor molecules and/or isoprenoids can similarly comprise the steps of: (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) that do not endogenously have a phosphoketolase, wherein the recombinant cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide; and (b) producing isoprenoid precursor molecules and/or isoprenoids, wherein the recombinant cells produce greater amounts of isoprenoid precursors and/or isoprenoids when compared to isoprenoids and/or isoprenoid precursor-producing cells that do not comprise the phosphoketolase polypeptide.

[0312] The instant methods for the production of isoprenoid precursor molecules and/or isoprenoids can produce at least 5% greater amounts of isoprenoid precursors and/or isoprenoids when compared to isoprenoids and/or isoprenoid precursor-producing recombinant cells that do not comprise a phosphoketolase polypeptide. Alternatively, the recombinant cells can produce greater than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% of isoprenoid precursors and/or isoprenoids, inclusive. In some aspects, the method of producing isoprenoid precursor molecules and/or isoprenoids further comprises a step of recovering the isoprenoid precursor molecules and/or isoprenoids.

[0313] Provided herein are methods of using any of the cells described above for enhanced isoprenoid and/or isoprenoid precursor molecule production. The production of isoprenoid precursor molecules and/or isoprenoids by the cells can be enhanced by the expression of one or more heterologous nucleic acids encoding phosphoketolase, and/or the mvaE and mvaS polypeptides from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis,* one or more heterologous nucleic acids encoding a lower MVA pathway polypeptide, and one or more heterologous nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide. As used herein, "enhanced" isoprenoid precursor and/or isoprenoid production refers to an increased cell productivity index (CPI) for isoprenoid precursor and/or isoprenoid production, an increased titer of isoprenoid precursors and/or isoprenoids, an increased mass yield of isoprenoid precursors and/or isoprenoids, and/or an increased specific productivity of isoprenoid precursors and/or isoprenoids by the cells described by any of the compositions and methods described herein compared to cells which do not have one or more

heterologous nucleic acids encoding a phosphoketolase, a polyprenyl pyrophosphate synthase polypeptide, a lower MVA pathway polypeptide(s), the mvaE and mvaS polypeptides from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*. The production of isoprenoid precursor molecules and/or isoprenoids can be enhanced by about 5% to about 1,000,000 folds. The production of isoprenoid precursor molecules and/or isoprenoids can be enhanced by about 10% to about 1,000,000 folds (*e.g.*, about 1 to about 500,000 folds, about 1 to about 50,000 folds, about 1 to about 5,000 folds, about 1 to about 1,000 folds, about 1 to about 500 folds, about 1 to about 100 folds, about 1 to about 50 folds, about 5 to about 100,000 folds, about 5 to about 10,000 folds, about 5 to about 1,000 folds, about 5 to about 500 folds, about 5 to about 100 folds, about 10 to about 50,000 folds, about 50 to about 10,000 folds, about 100 to about 5,000 folds, about 200 to about 1,000 folds, about 50 to about 500 folds, or about 50 to about 200 folds) compared to the production of isoprenoid precursor molecules and/or isoprenoids by cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. In certain embodiments described herein, the methods comprise recombinant host cells that have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprenoids and/or isoprenoid-precursors as compared to the production of isoprenoids and/or isoprenoid-precursors by isoprenoids and/or isoprenoid-precursors-producing cells that do not express one or more heterologous nucleic acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production.

[0314] The production of isoprenoid precursor molecules and/or isoprenoids can also enhanced by the methods described herein by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 20 folds, 50 folds, 100 folds, 200 folds, 500 folds, 1000 folds, 2000 folds, 5000 folds, 10,000 folds, 20,000 folds, 50,000 folds, 100,000 folds, 200,000 folds, 500,000 folds, or 1,000,000 folds compared to the production of isoprenoid precursor molecules and/or isoprenoids by isoprenoid precursors and/or isoprenoid-producing cells without the expression of one or more heterologous nucleic acids encoding a phosphoketolase polypeptide. In certain embodiments described herein, the methods comprise recombinant host cells that have been further engineered to increased carbon flux to MVA production thereby providing enhanced production of isoprenoids and/or isoprenoid-precursors as compared to the production of isoprenoids and/or isoprenoid-precursors by isoprenoids and/or isoprenoid-precursors-producing cells that do not express one or more heterologous nucleic

acids encoding phosphoketolase peptide and which have not been engineered for increased carbon flux to mevalonate production.

[0315] In addition, more specific cell culture conditions can be used to culture the cells in the methods described herein. For example, in some aspects, the method for the production of isoprenoid precursor molecules and/or isoprenoids comprises the steps of (a) culturing recombinant cells (including, but not limited to, *E. coli* cells) which comprise a heterologous nucleic acid which encodes a phosphoketolase polypeptide and that do not endogenously have an *mvaE* gene and an *mvaS* gene from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis* in minimal medium at 34°C, wherein the recombinant cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* on a low to medium copy plasmid and under the control of a strong promoter; and (b) producing isoprenoid precursor molecules and/or isoprenoids . In some aspects, the methods further comprise a step of recovering the isoprenoid precursor molecules and/or isoprenoids. In some aspects, wherein the recombinant cells heterologously express one or more copies of a gene encoding a phosphoketolase polypeptide from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.*

Vectors

[0316] Suitable vectors can be used for any of the compositions and methods described herein. For example, suitable vectors can be used to optimize the expression of one or more copies of a gene encoding a phosphoketolase, an upper MVA pathway polypeptide including, but not limited to, mvaE and an mvaS polypeptide, a lower MVA pathway polypeptide, an isoprene synthase, or a polyprenyl pyrophosphate synthase in a particular host cell (e.g., E. coli). In some aspects, the vector contains a selective marker. Examples of selectable markers include, but are not limited to, antibiotic resistance nucleic acids (*e.g.*, kanamycin, ampicillin, carbenicillin, gentamicin, hygromycin, phleomycin, bleomycin, neomycin, or chloramphenicol) and/or nucleic acids that confer a metabolic advantage, such as a nutritional advantage on the

host cell. In some aspects, one or more copies of a phosphoketolase, an upper MVA pathway polypeptide including, but not limited to, mvaE and an mvaS polypeptide, a lower MVA pathway polypeptide, an mvaE and an mvaS nucleic acid from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus*, and/or *E. faecalis*, an isoprene synthase, or a polyprenyl pyrophosphate synthase nucleic acid(s) integrate into the genome of host cells without a selective marker.

[0317] Any one of the vectors characterized herein or used in the Examples of the present disclosure can be used in the present invention.

Transformation methods

[0318] Nucleic acids encoding one or more copies of a phosphoketolase, an upper MVA pathway polypeptide including, but not limted to, mvaE and an mvaS polypeptide, a lower MVA pathway polypeptide, and/or lower MVA pathway polypeptides can be inserted into a cell using suitable techniques. Additionally, isoprene synthase, IDI, DXP pathway, and/or polyprenyl pyrophosphate synthase nucleic acids or vectors containing them can be inserted into a host cell (*e.g.*, a plant cell, a fungal cell, a yeast cell, or a bacterial cell described herein) using standard techniques for introduction of a DNA construct or vector into a host cell, such as transformation, electroporation, nuclear microinjection, transduction, transfection (*e.g.*, lipofection mediated or DEAE-Dextrin mediated transfection or transfection using a recombinant phage virus), incubation with calcium phosphate DNA precipitate, high velocity bombardment with DNA-coated microprojectiles, and protoplast fusion. General transformation techniques are known in the art (*See, e.g., Current Protocols in Molecular Biology* (F. M. Ausubel *et al.* (eds.) Chapter 9, 1987; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual,* 2nd ed., Cold Spring Harbor, 1989; and Campbell *et al.*, *Curr. Genet.* 16:53-56, 1989). The introduced nucleic acids can be integrated into chromosomal DNA or maintained as extrachromosomal replicating sequences. Transformants can be selected by any method known in the art. Suitable methods for selecting transformants are described in International Publication No. WO 2009/076676, U.S. Patent Publ. No. 2009/0203102, WO 2010/003007, US Publ. No. 2010/0048964, WO 2009/132220, and US Publ. No. 2010/0003716.

Exemplary host cells

[0319] One of skill in the art will recognize that expression vectors are designed to contain certain components which optimize gene expression for certain host strains. Such optimization components include, but are not limited to origin of replication, promoters, and enhancers. The vectors and components referenced herein are described for exemplary purposes and are not meant to narrow the scope of the invention.

[0320] Any cell or progeny thereof that can be used to heterologously express genes can be used to express one or more a phosphoketolase isolated from *Lactobacillus reuteri, Bifidobacterium longum, Ferrimonas balearica, Pedobactor saltans, Streptomyces griseus,* and/or *Nocardiopsis dassonvillei* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides, isoprene synthase, IDI, DXP pathway polypeptide(e), and/or polyprenyl pyrophosphate synthase polypeptides. In some embodiments, the host cell is a gram-positive bacterium. Non-limiting examples include strains of *Streptomyces* (e.g., *S. lividans, S. coelicolor, or S. griseus*), Bacillus, Listeria (e.g., *L. monocytogenes*) or Lactobacillus (e.g., *L. spp*). In some embodiments, the source organism is a gram-negative bacterium, such as *E. coli, Pseudomonas sp, or H. pylori.*

[0321] Bacteria cells, including gram positive or gram negative bacteria can be used to express any of the heterologous genes described above. In particular, the *mvaE* and *mvaS* genes can be expressed in any one of *P. citrea, B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. clausii, B. halodurans, B. megaterium, B. coagulans, B. circulans, B. lautus, B. thuringiensis, S. albus, S. lividans, S. coelicolor, S. griseus, Pseudomonas sp.,* and *P. alcaligenes* cells.

[0322] There are numerous types of anaerobic cells that can be used as host cells in the compositions and methods of the present invention. In one aspect of the invention, the cells described in any of the compositions or methods described herein are obligate anaerobic cells and progeny thereof. Obligate anaerobes typically do not grow well, if at all, in conditions where oxygen is present. It is to be understood that a small amount of oxygen may be present, that is, there is some tolerance level that obligate anaerobes have for a low level of oxygen. In one aspect, obligate anaerobes engineered to produce mevalonate, isoprenoid precursors, isoprene, and isoprenoids can serve as host cells for any of the methods and/or compositions

described herein and are grown under substantially oxygen-free conditions, wherein the amount of oxygen present is not harmful to the growth, maintenance, and/or fermentation of the anaerobes.

[0323] In another aspect of the invention, the host cells described and/or used in any of the compositions or methods described herein are facultative anaerobic cells and progeny thereof. Facultative anaerobes can generate cellular ATP by aerobic respiration (e.g., utilization of the TCA cycle) if oxygen is present. However, facultative anaerobes can also grow in the absence of oxygen. This is in contrast to obligate anaerobes which die or grow poorly in the presence of greater amounts of oxygen. In one aspect, therefore, facultative anaerobes can serve as host cells for any of the compositions and/or methods provided herein and can be engineered to produce mevalonate, isoprenoid precursors, isoprene, and isoprenoids. Facutative anerobic host cells can be grown under substantially oxygen-free conditions, wherein the amount of oxygen present is not harmful to the growth, maintenance, and/or fermentation of the anaerobes, or can be alternatviely grown in the presence of greater amounts of oxygen.

[0324] The host cell can additionally be a filamentous fungal cell and progeny thereof. (*See, e.g.,* Berka & Barnett, *Biotechnology Advances,* (1989), 7(2):127-154). In some aspects, the filamentous fungal cell can be any of *Trichoderma longibrachiatum, T. viride, T. koningii, T. harzianum*, Penicillium sp., *Humicola insolens, H. lanuginose, H. grisea, Chrysosporium sp., C. lucknowense, Gliocladium sp., Aspergillus sp.*, such as *A. oryzae, A. niger, A sojae, A. japonicus, A. nidulans*, or *A. awamori, Fusarium sp.,* such as *F. roseum, F. graminum F. cerealis, F. oxysporuim*, or *F. venenatum, Neurospora sp.,*such as *N. crassa, Hypocrea sp., Mucor sp.,*such as *M. miehei, Rhizopus sp.* or *Emericella sp.* In some aspects, the fungus is *A. nidulans, A. awamori, A. oryzae, A. aculeatus, A. niger, A. japonicus, T. reesei, T. viride, F. oxysporum,* or *F. solani.* In certain embodiments, plasmids or plasmid components for use herein include those described in U.S. patent pub. No. US 2011/0045563.

[0325] The host cell can also be a yeast, such as *Saccharomyces sp., Schizosaccharomyces sp., Pichia sp.,* or Candida *sp.* In some aspects, the *Saccharomyces sp.* is *Saccharomyces cerevisiae* (See, e.g., Romanos et al., *Yeast,* (1992), 8(6):423-488). In certain embodiments, plasmids or plasmid components for use herein include those described in U.S. pat. No, 7,659,097 and U.S. patent pub. No. US 2011/0045563.

[0326] The host cell can additionally be a species of algae, such as a green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, or dinoflagellates. (See, e.g., Saunders & Warmbrodt, "*Gene Expression in Algae and Fungi, Including Yeast*," (1993), National Agricultural Library, Beltsville, MD). In certain embodiments, plasmids or plasmid components for use herein include those described in U.S. Patent Pub. No. US 2011/0045563. In some aspects, the host cell is a cyanobacterium, such as cyanobacterium classified into any of the following groups based on morphology: *Chlorococcales, Pleurocapsales, Oscillatoriales, Nostocales,* or *Stigonematales* (See, e.g., Lindberg et al., Metab. Eng., (2010) 12(1):70-79). In certain embodiments, plasmids or plasmid components for use herein include those described in U.S. patent pub. No. US 2010/0297749; US 2009/0282545 and Intl. Pat. Appl. No. WO 2011/034863.

[0327] *E. coli* host cells can be used to express one or more phosphoketolase enzymes from from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* along with one or more heterologous nucleic acids encoding one or more MVA pathway polypeptides, isoprene synthase, IDI, DXP pathway polypeptide(e), and/or polyprenyl pyrophosphate synthase polypeptides. In one aspect, the host cell is a recombinant cell of an *Escherichia coli* (*E. coli*) strain, or progeny thereof, capable of producing mevalonate that expresses one or more nucleic acids encoding phosphoketolase from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides. The *E. coli* host cells can produce mevalonate in amounts, peak titers, and cell productivities greater than that of the same cells lacking one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides from *Clostridium*

acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides. In addition, the one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptide from *Clostridium acetobutylicum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus paraplantarum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Enterococcus gallinarum, Gardnerella vaginalis, Ferrimonas balearica, Mucilaginibacter paludis, Nostoc punctiforme, Nostoc punctiforme PCC 73102, Pantoea, Pedobactor saltans, Rahnella aquatilis, Rhodopseudomonas palustris, Streptomyces griseus, Streptomyces avermitilis, Nocardiopsis dassonvillei, and/or Thermobifida fusca* along with one or more heterologous nucleic acids expressing one or more MVA pathway peptides in *E. coli* can be chromosomal copies (e.g., integrated into the *E. coli* chromosome). In other aspects, the *E. coli* cells are in culture. In some aspects the one or more phosphoketolase enzymes is from *Clostridium acetobutylicum, Bifidobacterium longum,* and/or *Enterococcus gallinarum.* In any aspects, the one ore more phosphoketolase enzymes are any phosphoketlase enzymes as disclosed herein.

Exemplary Host Cell Modifications

Citrate Synthase Pathway

[0328] Citrate synthase catalyzes the condensation of oxaloacetate and acetyl-CoA to form citrate, a metabolite of the tricarboxylic acid (TCA) cycle (Ner, S. et al. 1983. *Biochemistry*, 22: 5243-5249; Bhayana, V. and Duckworth, H. 1984. Biochemistry 23: 2900-2905). In *E. coli*, this enzyme, encoded by *gltA*, behaves like a trimer of dimeric subunits. The hexameric form allows the enzyme to be allosterically regulated by NADH. This enzyme has been widely studied (Wiegand, G., and Remington, S. 1986. Annual Rev. Biophysics Biophys. Chem.15: 97-117; Duckworth et al. 1987. Biochem Soc Symp. 54:83-92; Stockell, D. et al. 2003. J. Biol. Chem. 278: 35435-43; Maurus, R. et al. 2003. Biochemistry. 42:5555-5565). To avoid allosteric inhibition by NADH, replacement by or supplementation with the *Bacillus*

subtilis NADH-insensitive citrate synthase has been considered (Underwood et al. 2002. Appl. Environ. Microbiol. 68:1071-1081; Sanchez et al. 2005. Met. Eng. 7:229-239).

[0329] The reaction catalyzed by citrate synthase is directly competing with the thiolase catalyzing the first step of the mevalonate pathway, as they both have acetyl-CoA as a substrate (Hedl et al. 2002. J. Bact. 184:2116-2122). Therefore, one of skill in the art can modulate citrate synthase expression (e.g., decrease enzyme activity) to allow more carbon to flux into the mevalonate pathway, thereby increasing the eventual production of mevalonate, isoprene and isoprenoids. Decrease of citrate synthase activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some aspects, the activity of citrate synthase is modulated by decreasing the activity of an endogenous citrate synthase gene. This can be accomplished by chromosomal replacement of an endogenous citrate synthase gene with a transgene encoding an NADH-insensitive citrate synthase or by using a transgene encoding an NADH-insensitive citrate synthase that is derived from *Bacillus subtilis*. The activity of citrate synthase can also be modulated (e.g., decreased) by replacing the endogenous citrate synthase gene promoter with a synthetic constitutively low expressing promoter. The gene encoding citrate synthase can also be deleted. The decrease of the activity of citrate synthase can result in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have decreased expression of citrate synthase. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of citrate synthase (gltA). Activity modulation (e.g., decreased) of citrate synthase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a citrate synthase isozyme.

Pathways involving Phosphotransacetylase and/or Acetate Kinase

[0330] Phosphotransacetylase ((encoded in *E. coli* by (i) *pta* (Shimizu et al. 1969. Biochim. Biophys. Acta 191: 550-558 or (ii) *eutD* (Bologna et al. 2010. J of Microbiology.

48:629-636) catalyzes the reversible conversion between acetyl-CoA and acetyl phosphate (acetyl-P), while acetate kinase (encoded in *E. coli* by *ackA*) (Kakuda, H. et al. 1994. J. Biochem. 11:916-922) uses acetyl-P to form acetate. These genes can be transcribed as an operon in *E. coli*. Together, they catalyze the dissimilation of acetate, with the release of ATP. Thus, it is possible to increase the amount of acetyl-P going towards acetyl-CoA by enhancing the activity of phosphotransacetylase. In certain embodiments, enhancement is achieved by placing an upregulated promoter upstream of the gene in the chromosome, or to place a copy of the gene behind an adequate promoter on a plasmid. In order to decrease the amount of acetyl-coA going towards acetate, the activity of acetate kinase gene (e.g., the endogenous acetate kinase gene) can be decreased or attenuated. In certain embodiments, attenuation is achieved by deleting acetate kinase (ackA). This is done by replacing the gene with a chloramphenicol cassette followed by looping out of the cassette. In some aspects, the activity of acetate kinase is modulated by decreasing the activity of an endogenous acetate kinase. This can be accomplished by replacing the endogenous acetate kinase gene promoter with a synthetic constitutively low expressing promoter. In certain embodiments, it the attenuation of the acetated kinase gene should be done disrupting the expression of the phosphotransacetylase (*pta*) gene. Acetate is produced by *E. coli* for a variety of reasons (Wolfe, A. 2005. Microb. Mol. Biol. Rev. 69:12-50). Without being bound by theory, deletion of ackA could result in decreased carbon being diverted into acetate production (since ackA use acetyl-CoA) and thereby increase the yield of mevalonate, isoprenoid precursors, isoprene and/or isoprenoids.

[0331] In some aspects, the recombinant cells described herein produce decreased amounts of acetate in comparison to cells that do not have attenuated endogenous acetate kinase gene expression or enhanced phosphotransacetylase. Decrease in the amount of acetate produced can be measured by routine assays known to one of skill in the art. The amount of acetate reduction is at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% as compared when no molecular manipulations are done to the endogenous acetate kinase gene expression or phosphotransacetylase gene expression.

[0332] The activity of phosphotransacetylase (pta and/or eutD) can be increased by other molecular manipulations of the enzymes. The increase of enzyme activity can be and increase in any amount of specific activity or total activity as compared to when no manipulation

has been effectuated. In some instances, the increase of enzyme activity is increased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In one embodiment the activity of pta is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of phosphotransacetylase (pta and/or eutD). Activity modulation (e.g., increased) of phosphotransacetylase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of a phosphotransacetylase (pta and/or eutD) isozyme.

[0333] The activity of acetate kinase (ackA) can also be decreased by other molecular manipulations of the enzymes. The decrease of enzyme activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of acetate kinase (ackA). Activity modulation (e.g., decreased) of acetate kinase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a acetate kinase isozyme.

[0334] In some cases, attenuating the activity of the endogenous acetate kinase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have attenuated endogenous acetate gene expression.

Pathways Involving Lactate Dehydrogenase

[0335] In *E. coli*, D-Lactate is produced from pyruvate through the enzyme lactate dehydrogenase (encoded by *ldhA* – Figure 1) (Bunch, P. et al. 1997. Microbiol. 143:187-195). Production of lactate is accompanied with oxidation of NADH, hence lactate is produced when oxygen is limited and cannot accommodate all the reducing equivalents. Thus, production of lactate could be a source for carbon consumption. As such, to improve carbon flow through to mevalonate production (and isoprene, isoprenoid precursor and isoprenoids production, if desired), one of skill in the art can modulate the activity of lactate dehydrogenase, such as by decreasing the activity of the enzyme.

[0336] Accordingly, in one aspect, the activity of lactate dehydrogenase can be modulated by attenuating the activity of an endogenous lactate dehydrogenase gene. Such attenuation can be achieved by deletion of the endogenous lactate dehydrogenase gene. Other ways of attenuating the activity of lactate dehydrogenase gene known to one of skill in the art may also be used. By manipulating the pathway that involves lactate dehydrogenase, the recombinant cell produces decreased amounts of lactate in comparison to cells that do not have attenuated endogenous lactate dehydrogenase gene expression. Decrease in the amount of lactate produced can be measured by routine assays known to one of skill in the art. The amount of lactate reduction is at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% as compared when no molecular manipulations are done.

[0337] The activity of lactate dehydrogenase can also be decreased by other molecular manipulations of the enzyme. The decrease of enzyme activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%.

[0338] Accordingly, in some cases, attenuation of the activity of the endogenous lactate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have attenuated endogenous lactate dehydrogenase gene expression.

Pathways involving glyceraldehyde 3-phosphate

[0339] Glyceraldehyde 3-phosphate dehydrogenase (*gapA* and/or *gapB*) is a crucial enzyme of glycolysis catalyzes the conversion of glyceraldehyde 3-phosphate into 1,3-biphospho-D-glycerate (Branlant G. and Branlant C. 1985. Eur. J. Biochem. 150:61-66).

[0340] In order to direct carbon towards the phosphoketolase enzyme, glyceraldehyde 3-phosphate dehydrogenase expression can be modulated (e.g., decrease enzyme activity) to allow more carbon to flux towards fructose 6-phosphate and xylulose 5-phosphate, thereby increasing the eventual production of mevalonate, isoprene and isoprenoids. Decrease of glyceraldehyde 3-phosphate dehydrogenase activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%. Or 100%. In some aspects, the activity of glyceraldehyde 3-phosphate dehydrogenase is modulated by decreasing the activity of an endogenous glyceraldehyde 3-phosphate dehydrogenase. This can be accomplished by replacing the endogenous glyceraldehyde 3-phosphate dehydrogenase gene promoter with a synthetic constitutively low expressing promoter. The gene encoding glyceraldehyde 3-phosphate dehydrogenase can also be deleted. The gene encoding glyceraldehyde 3-phosphate dehydrogenase can also be replaced by a *Bacillus* enzyme catalyzing the same reaction but producing NADPH rather than NADH. The decrease of the activity of glyceraldehyde 3-phosphate dehydrogenase can result in more carbon flux into the mevalonate-dependent biosynthetic pathway in comparison to cells that do not have decreased expression of glyceraldehyde 3-phosphate dehydrogenase. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of glyceraldehyde 3-phosphate dehydrogenase (*gapA* and/or *gapB*). Activity modulation (e.g., decreased) of glyceraldehyde 3-phosphate dehydrogenase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a glyceraldehyde 3-phosphate dehydrogenase (*gapA* and/or *gapB*) isozyme.

Pathways involving the Entner-Doudoroff pathway

[0341] The Entner-Doudoroff (ED) pathway is an alternative to the Emden-Meyerhoff-Parnass (EMP –glycolysis) pathway. Some organisms, like *E. coli*, harbor both the ED and EMP pathways, while others have only one or the other. *Bacillus* subtilis has only the EMP pathway, while *Zymomonas mobilis* has only the ED pathway (Peekhaus and Conway. 1998. J. Bact. 180:3495-3502; Stulke and Hillen. 2000. Annu. Rev. Microbiol. 54, 849–880; Dawes et al. 1966. Biochem. J. 98:795-803). Fructose bisphophate aldolase (fba, fbaA, fbaB, and/or fbaC) interacts with the Entner-Doudoroff pathway and reversibly catalyzes the conversion of fructose 1,6-bisphosphate into dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP) (Baldwin S.A., et. al., Biochem J. (1978) 169(3):633-41).

[0342] Phosphogluconate dehydratase (edd) removes one molecule of H_2O from 6-phospho-D-gluconate to form 2-dehydro-3-deoxy-D-gluconate 6-phosphate, while 2-keto-3-deoxygluconate 6-phosphate aldolase (eda) catalyzes an aldol cleavage (Egan et al. 1992. J. Bact. 174:4638-4646). The two genes are in an operon.

[0343] Metabolites that can be directed into the phosphoketolase pathway can also be diverted into the ED pathway. To avoid metabolite loss to the ED-pathway, phosphogluconate dehydratase gene (e.g., the endogenous phosphogluconate dehydratase gene) and/or an 2-keto-3-deoxygluconate 6-phosphate aldolase gene (e.g., the endogenous 2-keto-3-deoxygluconate 6-phosphate aldolase gene) activity is attenuated. One way of achieving attenuation is by deleting phosphogluconate dehydratase (edd) and/or 2-keto-3-deoxygluconate 6-phosphate aldolase (eda). This can be accomplished by replacing one or both genes with a chloramphenicol or kanamycin cassette followed by looping out of the cassette. Without these enzymatic activities, more carbon can flux through the phosphoketolase enzyme, thus increasing the yield of mevalonate, isoprene or isoprenoids.

[0344] The activity of phosphogluconate dehydratase (edd) and/or 2-keto-3-deoxygluconate 6-phosphate aldolase (eda) can also be decreased by other molecular manipulations of the enzymes. The decrease of enzyme activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of enzyme activity is decreased by at least about 1%, 2%, 3%,

4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%.

[0345] In some cases, attenuating the activity of the endogenous phosphogluconate dehydratase gene and/or the endogenous 2-keto-3-deoxygluconate 6-phosphate aldolase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have attenuated endogenous phosphogluconate dehydratase gene and/or endogenous acetate kinase2-keto-3-deoxygluconate 6-phosphate aldolase gene expression.

[0346] Metabolites that can be directed into the phosphoketolase pathway can also be diverted into the ED pathway or EMP pathway. To avoid metabolite loss and to increase fructose-6-phosphate (F6P) concentration, fructose bisphophate aldolase (e.g., the endogenous fructose bisphophate aldolase) activity is attenuated. In some cases, attenuating the activity of the endogenous fructose bisphophate aldolase (fba, fbaA, fbaB, and/or fbaC) gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have attenuated endogenous fructose bisphophate aldolase (fba, fbaA, fbaB, and/or fbaC) gene expression. In some aspects, attenuation is achieved by deleting fructose bisphophate aldolase (fba, fbaA, fbaB, and/or fbaC). Deletion can be accomplished by replacing the gene with a chloramphenicol or kanamycin cassette followed by looping out of the cassette. In some aspects, the activity of fructose bisphophate aldolase is modulated by decreasing the activity of an endogenous fructose bisphophate aldolase. This can be accomplished by replacing the endogenous fructose bisphophate aldolase gene promoter with a synthetic constitutively low expressing promoter. Without these enzymatic activities, more carbon can flux through the phosphoketolase enzyme, thus increasing the yield of mevalonate, isoprene or isoprenoids. The activity of fructose bisphophate aldolase can also be decreased by other molecular manipulations of the enzyme. The decrease of enzyme activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of fructose bisphophate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*). Activity

modulation (e.g., decreased) of fructose bisphophate aldolase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a fructose bisphophate aldolase isozyme.

Pathways involving the oxidative branch of the pentose phosphate pathway

[0347] *E. coli* uses the pentose phosphate pathway to break down hexoses and pentoses and to provide cells with intermediates for various anabolic pathways. It is also a major producer of NADPH. The pentose phosphate pathway is composed from an oxidative branch (with enzymes like glucose 6-phosphate 1-dehydrogenase (*zwf*), 6-phosphogluconolactonase (*pgl*) or 6-phosphogluconate dehydrogenase (*gnd*)) and a non-oxidative branch (with enzymes such as transketolase (*tktA* and/or *tktB*), transaldolase (*talA* or *talB*), ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase, ribose-5-phosphate isomerase (*rpiA and/or rpiB*) and/or ribulose-5-phosphate 3-epimerase (*rpe*)) (Sprenger. 1995. Arch. Microbiol.164:324-330).

[0348] In order to direct carbon towards the phosphoketolase enzyme, the non-oxidative branch of the pentose phosphate pathway (transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase, ribose-5-phosphate isomerase A, ribose-5-phosphate isomerase B, and/or ribulose-5-phosphate 3-epimerase) expression can be modulated (e.g., increase enzyme activity) to allow more carbon to flux towards fructose 6-phosphate and xylulose 5-phosphate, thereby increasing the eventual production of mevalonate, isoprene and isoprenoids. Increase of transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase activity can be any amount of increase of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the enzyme activity is increased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some aspects, the activity of transketolase, transaldolase , ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase is modulated by increasing the activity of an endogenous transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase. This can be accomplished by replacing the endogenous transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase gene promoter with a synthetic constitutively

high expressing promoter. The genes encoding transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can also be cloned on a plasmid behind an appropriate promoter. The increase of the activity of transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can result in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have increased expression of transketolase, transaldolase , ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase.

[0349] In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of transketolase (*tktA* and/or *tktB*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of transketolase (*tktA* and/or *tktB*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of transaldolase (*talA* or *talB*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of ribose-5-phosphate isomerase (*rpiA and/or rpiB*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of ribulose-5-phosphate 3-epimerase (*rpe*). Activity modulation (e.g., decreased or increased) of glucose 6-phosphate 1-dehydrogenase (*zwf*), 6-phosphogluconolactonase (*pgl*), 6-phosphogluconate dehydrogenase (*gnd*), transketolase (*tktA* and/or *tktB*), transaldolase (*talA* or *talB*), ribulose-5-phosphate-epimerase, ribose-5-phosphate epimerase, ribose-5-phosphate isomerase (*rpiA and/or rpiB*) and/or ribulose-5-phosphate 3-epimerase (*rpe*) isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to incrrease the activity of a glucose 6-phosphate 1-dehydrogenase (*zwf*) isozyme. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase

polypeptides as disclosed herein and further engineered to increase the activity of a transketolase (*tktA* and/or *tktB*) isozyme. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to deacrease the activity of a transketolase (*tktA* and/or *tktB*) isozyme. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of a transaldolase (*talA* or *talB*) isozyme. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of a ribose-5-phosphate isomerase (*rpiA and/or rpiB*) isozyme. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of a ribulose-5-phosphate 3-epimerase (*rpe*) isozyme.

[0350] In order to direct carbon towards the phosphoketolase enzyme, glucose 6-phosphate 1-dehydrogenase can be modulated (e.g., decrease enzyme activity). In some aspects, the activity of glucose 6-phosphate 1-dehydrogenase (*zwf*) (e.g., the endogenous glucose 6-phosphate 1-dehydrogenase gene) can be decreased or attenuated. In certain embodiments, attenuation is achieved by deleting glucose 6-phosphate 1-dehydrogenase. In some aspects, the activity of glucose 6-phosphate 1-dehydrogenase is modulated by decreasing the activity of an endogenous glucose 6-phosphate 1-dehydrogenase. This can be accomplished by replacing the endogenous glucose 6-phosphate 1-dehydrogenase gene promoter with a synthetic constitutively low expressing promoter. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of glucose 6-phosphate 1-dehydrogenase (*zwf*). Activity modulation (e.g., decreased) of glucose 6-phosphate 1-dehydrogenase isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a glucose 6-phosphate 1-dehydrogenase isozyme.

Pathways involving phosphofructokinase

[0351] Phosphofructokinase is a crucial enzyme of glycolysis which catalyzes the phosphorylation of fructose 6-phosphate. E. coli has two isozymes encoded by pfkA and pfkB. Most of the phosphofructokinase activity in the cell is due to pfkA (Kotlarz et al. 1975 Biochim. Biophys. Acta 381:257-268).

[0352] In order to direct carbon towards the phosphoketolase enzyme, phosphofructokinase expression can be modulated (e.g., decrease enzyme activity) to allow more carbon to flux towards fructose 6-phosphate and xylulose 5-phosphate, thereby increasing the eventual production of mevalonate, isoprene and isoprenoids. Decrease of phosphofructokinase activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of enzyme activity is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%. Or 100%. In some aspects, the activity of phosphofructokinase is modulated by decreasing the activity of an endogenous phosphofructokinase. This can be accomplished by replacing the endogenous phosphofructokinase gene promoter with a synthetic constitutively low expressing promoter. The gene encoding phosphofructokinase can also be deleted. The decrease of the activity of phosphofructokinase can result in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have decreased expression of phosphofructokinase.

[0353] In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of fructose 6-phosphate (*pfkA* and/or *pfkB*). Activity modulation (e.g., decreased) of fructose 6-phosphate isozymes is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of a fructose 6-phosphate isozyme.

Pathways Involving Pyruvate Dehydrogenase Complex

[0354] The pyruvate dehydrogenase complex, which catalyzes the decarboxylation of pyruvate into acetyl-CoA, is composed of the proteins encoded by the genes aceE, aceF and lpdA. Transcription of those genes is regulated by several regulators. Thus, one of skill in the art can increase acetyl-CoA by modulating the activity of the pyruvate dehydrogenase complex. Modulation can be to increase the activity and/or expression (e.g., constant expression) of the pyruvate dehydrogenase complex. This can be accomplished by different ways, for example, by placing a strong constitutive promoter, like PL.6 (aattcatataaaaaacatacagataaccatctgcggtgataaattatctctggcggtgttgacataaataccactggcggtgatactgagcac atcagcaggacgcactgaccaccatgaaggtg - lambda promoter, GenBank NC_001416, SEQ ID NO:14), in front of the operon or using one or more synthetic constitutively expressing promoters.

[0355] Accordingly, in one aspect, the activity of pyruvate dehydrogenase is modulated by increasing the activity of one or more enzymes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase. It is understood that any one, two or three of the genes encoding these enzymes can be manipulated for increasing activity of pyruvate dehydrogenase. In another aspect, the activity of the pyruvate dehydrogenase complex can be modulated by attenuating the activity of an endogenous pyruvate dehydrogenase complex repressor, further detailed below. The activity of an endogenous pyruvate dehydrogenase complex repressor can be attenuated by deletion of the endogenous pyruvate dehydrogenase complex repressor gene.

[0356] In some cases, one or more genes encoding the pyruvate dehydrogenase complex are endogenous genes. Another way to increase the activity of the pyruvate dehydrogenase complex is by introducing into the cell one or more heterologous nucleic acids encoding one or more polypeptides from the group consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

[0357] By using any of these methods, the recombinant cells can produce increased amounts of acetyl Co-A in comparison to cells wherein the activity of pyruvate dehydrogenase is not modulated. Modulating the activity of pyruvate dehydrogenase can result in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to cells that do not have modulated pyruvate dehydrogenase expression.

Pathways involving the phosphotransferase system

[0358] The phosphoenolpyruvate dependent phosphotransferase system (PTS) is a multicomponent system that simultaneously transports and phosphorylates its carbohydrate substrates across a membrane in a process that is dependent on energy provided by the glycolytic intermediate phosphoenolpyruvate (PEP). The genes that regulate the PTS are mostly clustered in operons. For example, the pts operon (ptsHIcrr) of Escherichia coli is composed of the ptsH, ptsI and crr genes coding for three proteins central to the phosphoenolpyruvate dependent phosphotransferase system (PTS), the HPr (*ptsH*), enzyme I (*ptsI*) and EIIIGlc (*crr*) proteins. These three genes are organized in a complex operon in which the major part of expression of the distal gene, crr, is initiated from a promoter region within ptsI. In addition to the genes of the pts operon, *ptsG* encodes the glucose-specific transporter of the phosphotransferase system, ptsG Transcription from this promoter region is under the positive control of catabolite activator protein (CAP)-cyclic AMP (cAMP) and is enhanced during growth in the presence of glucose (a PTS substrate). Furthermore, the ppsA gene encodes for phosphoenolpyruvate synthetase for the production of phosphoenolpyruvate (PEP) which is required for activity of the phosphotransferase system (PTS). Carbon flux is directed by the phosphoenolpyruvate synthetase through the pyruvate dehydrogenase pathway or the PTS pathway. See Postma, P.W., et al., Microbiol Rev. (1993), 57(3):543-94) which is incorporated herein by reference in its entirety.

[0359] In certain embodiments described herein, the down regulation (e.g. attenuation) of the pts operon can enhance acetate utilization by the host cells. The down regulation of PTS operon activity can be any amount of reduction of specific activity or total activity as compared to when no manipulation has been effectuated. In some instances, the decrease of activity of the complex is decreased by at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, attenuation is achieved by deleting the pts operon. In some aspects, the activity of the PTS system is modulated by decreasing the activity of an endogenous pts operon. This can be accomplished by replacing the endogenous promoter(s) within the pts operon with synthetic constitutively low expressing promoter(s). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed

herein and further engineered to decrease the activity of the pts operon. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of EI (*ptsI*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of $EIICB^{Glc}$ (*ptsG*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of $EIIA^{Glc}$ (*crr*). In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of HPr (*ptsH*). To decrease carbon loss through pyruvate dehydrogenase while increasing the PEP pool for glucose uptake, the activity of phosphoenolpyruvate synthetase (*ppsA*) can be increased. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to increase the activity of phosphoenolpyruvate synthetase (*ppsA*). In any further aspect of the invention, the PTS is downregulated and a glucose transport pathway is upregulated. A glucose transport pathway includes, but is not limited to, galactose (*galP*) and glucokinase (*glk*). In some embodiments, the pts operon is downregulated, the galactose (*galP*) gene is upregulated, and the glucokinase (*glk*) gene is upregulated. Activity modulation (e.g., decreased) of isozymes of the PTS is also contemplated herein. In any aspects of the invention, provided herein are recombinant cells comprising one or more heterologously expressed nucleic acids encoding phosphoketolase polypeptides as disclosed herein and further engineered to decrease the activity of PTS isozymes.

Pathways involving xylose utilization

[0360] In certain embodiments described herein, the utilization of xylose is desirable to convert suger derived from plant biomass into desired products, such as mevalonate, such as isoprenoid precursors, isoprene and/or isoprenoids. In some organisms, xylose utilization requires use of the pentose phosphate pathway for conversion to fructose-6-phosphate for metabolism. Organisms can be engineered for enhanced xylose utilization, either by

deactivating the catabolite repression by glucose, or by heterologous expression of genes from the xylose operon found in other organisms. The xylulose pathway can be engineered as described below to enhance production of mevalonate, isoprenoid precursors, isoprene and/or isoprenoids via the phosphoketolase pathway.

[0361] Enhancement of xylose uptake and conversion to xylulose-5-phosphate followed by direct entry into the phosphoketolase pathway would be a benefit. Without being bound by theory, this allows the carbon flux to bypass the pentose phosphate pathway (although some glyceraldehyde-3-phosphate may be cycled into PPP as needed). Enhanced expression of xyulokinase can be used to increase the overall production of xylulose-5-phosphate. Optimization of xyluokinase expression and activity can be used to enhance xylose utilization in a strain with a phosphoketolase pathway. The desired xyulokinase may be either the endogeneous host's enzyme, or any heterologous xyulokinase compatible with the host. In one embodiment, other components of the xylose operon can be overexpressed for increased benefit (e.g., xylose isomerase). In another embodiment, other xylose pathway enzymes (e.g. xylose reductase) may need to be attenuated (e.g., reduced or deleted activity).

[0362] Accordingly, the host cells engineered to have phosphoketolase enzymes as described herein can be further engineered to overexpress xylulose isomerase and/or xyulokinase, either the endocgenous forms or heterologous forms, to improve overall yield and productivity of mevalonate, isoprenoid precursors, isoprene and/or isoprenoids.

Pathways Involving Transaldolase and Transketolase Enzymes of Pentose Phosphate Pathway

[0363] Some microorganisms capable of anaerobic or heterofermentative growth incorporate a phosphoketolase pathway instead of or in addition to a glycolytic pathway. This pathway depends on the activity of the pentose phosphate pathway enzymes transaldolase and transketolase. Accordingly, the host cells engineered to have phosphoketolase enzymes as described herein can be further engineered to overexpress a transketolase and transaldolase, either the endogeneous forms or heterologous forms, to improve pathway flux, decrease the levels of potentially toxic intermediates, reduce the diversion of intermediates to non-productive pathways, and improve the overall yield and productivity of mevalonate, isoprenoid precursors, isoprene and/or isoprenoids.

Combinations of Mutations

[0364] It is understood that for any of the enzymes and/or enzyme pathways described herein, molecular manipulations that modulate any combination (two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen) of the enzymes and/or enzyme pathways described herein is expressly contemplated. For ease of the recitation of the combinations, citrate synthase (gltA) is designated as A, phosphotransacetylase (pta) is designated as B, acetate kinase (ackA) is designated as C, lactate dehydrogenase (ldhA) is designated as D, glyceraldehyde 3-phosphate dehydrogenase (gap) is designated as E, and pyruvate decarboxylase (aceE, aceF, and/or lpdA) is designated as F, phosphogluconate dehydratase (edd) is designated as G, 2-keto-3-deoxygluconate 6-phosphate aldolase (eda) is designated as H phosphofructokinase is designated as I, transaldolase is designated as J, transketolase is designated as K, ribulose-5-phosphate-epimerase is designated as L, ribose-5-phosphate epimerase is designated as M, xylukinase is designated as N, xylose isomerase is designated as O, and xylitol reductase is designated as P, ribose-5-phosphate isomerase (rpi) is designated as Q, D-ribulose-5-phosphate 3-epimerase (rpe) is designated as R, phosphoenolpyruvate synthetase (pps) is designated as S, fructose bisphosphate aldolase (fba) is designated as T, EI (ptsI) is designated as U, $EIICB^{Glc}$ (ptsG) is designated as V, $EIIA^{Glc}$ (crr) is designated as W, HPr (ptsH) is designated as X, galactose (galP) is designated as Y, glucokinase (glk) is designated as Z, glucose-6-phosphate dehydrogenase (zwf) is designated as AA. As discussed above, aceE, aceF, and/or lpdA enzymes of the pyruvate decarboxylase complex can be used singly, or two of three enzymes, or three of three enzymes for increasing pyruvate decarboxylase activity. Thus, any and all combination of enzymes designated as A-M herein is expressly contemplated as well as any and all combination of enzymes designated as A-AA. Furthermore, any combination described above can be used in combination with any of the enzymes and/or enzyme pathways described herein (e.g., phosphoketolase, MVA pathway polypeptides, isoprene synthase, DXP pathway polypeptides).

Other Regulators and Factors for Increased Production

[0365] Other molecular manipulations can be used to increase the flow of carbon towards mevalonate production. One method is to reduce, decrease or eliminate the effects of negative regulators for pathways that feed into the mevalonate pathway. For example, in some cases, the genes aceEF-lpdA are in an operon, with a fourth gene upstream pdhR. The gene

pdhR is a negative regulator of the transcription of its operon. In the absence of pyruvate, it binds its target promoter and represses transcription. It also regulates ndh and cyoABCD in the same way (Ogasawara, H. et al. 2007. J. Bact. 189:5534-5541). In one aspect, deletion of pdhR regulator can improve the supply of pyruvate, and hence the production of mevalonate, isoprenoid precursors, isoprene, and isoprenoids.

[0366] In other embodiments, any of the resultant strains described above can be further engineered to modulate the activity of the Entner-Doudoroff pathway. The gene coding for phosphogluconate dehydratase or aldolase can be attenuated or deleted. In other embodiments, any of the resultant strains described above may also be engineered to decrease or remove the activity of acetate kinase or citrate synthase. In other embodiments, any of the strains the resultant strain may also be engineered to decrease or remove the activity of phosphofructokinase. In other embodiments, any of the resultant strains described above may also be engineered to modulate the activity of glyceraldehyde-3-phosphate dehydrogenase. The activity of glyceraldehyde-3-phosphate dehydrogenase can be modulated by decreasing its activity. In other embodiments, the enzymes from the non-oxidative branch of the pentose phosphate pathway, such as transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can be overexpressed.

[0367] In other aspects, the host cells can be further engineered to increase intracellular acetyl-phospate concentrations by introducing heterologous nucleic acids encoding sedoheptulose-1,7-bisphosphatase/fructose-1,6 –bisphosphate aldolase and sedoheptulose-1,7-bisphosphatase/fructose-1,6–bisphosphate phosphatase. In certain embodiments, the host cells having these molecular manipulations can be combined with attenuated or deleted transaldolase (talB) and phosphofructokinase (pfkA and/or pfkB) genes, thereby allowing faster conversion of erythrose 4-phosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate into sedoheptulose 7-phosphate and fructose 1-phosphate (see Figure 5).

[0368] In other aspects, the introduction of 6-phosphogluconolactonase (PGL) into cells (such as various *E.* coli strains) which lack PGL can be used to improve production of mevalonate, isoprenoid precursors, isoprene, and isoprenoids. PGL may be introduced by introduction of the encoding gene using chromosomal integration or extra-chromosomal vehicles, such as plasmids.

[0369] In addition to the host cell (e.g., bacterial host cell) mutations for modulating various enzymatic pathways described herein that increases carbon flux towards mevalonate production, the host cells described herein comprise genes encoding phosphoketolase polypeptide, as well as other enzymes from the upper and lower MVA pathway, including but not limited to, the *mvaE* and *mvaS* gene products. Non-limiting examples of MVA pathway polypeptides include acetyl-CoA acetyltransferase (AA-CoA thiolase) polypeptides, 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) polypeptides, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) polypeptides, mevalonate kinase (MVK) polypeptides, phosphomevalonate kinase (PMK) polypeptides, diphosphomevalonte decarboxylase (MVD) polypeptides, phosphomevalonate decarboxylase (PMDC) polypeptides, isopentenyl phosphate kinase (IPK) polypeptides, IDI polypeptides, and polypeptides (*e.g.*, fusion polypeptides) having an activity of two or more MVA pathway polypeptides. MVA pathway polypeptides can include polypeptides, fragments of polypeptides, peptides, and fusions polypeptides that have at least one activity of an MVA pathway polypeptide. Exemplary MVA pathway nucleic acids include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of an MVA pathway polypeptide. Exemplary MVA pathway polypeptides and nucleic acids include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein.

[0370] Non-limiting examples of MVA pathway polypeptides which can be used are described in International Patent Application Publication No. WO2009/076676; WO2010/003007 and WO2010/148150

Exemplary Cell Culture Media

[0371] As used herein, the terms "minimal medium" or "minimal media" refer to growth media containing the minimum nutrients possible for cell growth, generally, but not always, without the presence of one or more amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids). Minimal medium typically contains: (1) a carbon source for bacterial growth; (2) various salts, which can vary among bacterial species and growing conditions; and (3) water. The carbon source can vary significantly, from simple sugars like glucose to more complex hydrolysates of other biomass, such as yeast extract, as discussed in more detail below. The salts generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the cells to synthesize proteins and nucleic acids. Minimal medium can also be

supplemented with selective agents, such as antibiotics, to select for the maintenance of certain plasmids and the like. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent cells lacking the resistance from growing. Medium can be supplemented with other compounds as necessary to select for desired physiological or biochemical characteristics, such as particular amino acids and the like.

[0372] Any minimal medium formulation can be used to cultivate the host cells. Exemplary minimal medium formulations include, for example, M9 minimal medium and TM3 minimal medium. Each liter of M9 minimal medium contains (1) 200 ml sterile M9 salts (64 g Na_2HPO_4-$7H_2O$, 15 g KH_2PO_4, 2.5 g NaCl, and 5.0 g NH_4Cl per liter); (2) 2 ml of 1 M $MgSO_4$ (sterile); (3) 20 ml of 20% (w/v) glucose (or other carbon source); and (4) 100 µl of 1 M $CaCl_2$ (sterile). Each liter of TM3 minimal medium contains (1) 13.6 g K_2HPO_4; (2) 13.6 g KH_2PO_4; (3) 2 g $MgSO_4$*$7H_2O$; (4) 2 g Citric Acid Monohydrate; (5) 0.3 g Ferric Ammonium Citrate; (6) 3.2 g $(NH_4)_2SO_4$; (7) 0.2 g yeast extract; and (8) 1 ml of 1000X Trace Elements solution; pH is adjusted to ~6.8 and the solution is filter sterilized. Each liter of 1000X Trace Elements contains: (1) 40 g Citric Acid Monohydrate; (2) 30 g $MnSO_4$*H_2O; (3) 10 g NaCl; (4) 1 g $FeSO_4$*$7H_2O$; (4)1 g $CoCl_2$*$6H_2O$; (5) 1 g $ZnSO_4$*$7H_2O$; (6) 100 mg $CuSO_4$*$5H_2O$; (7) 100 mg H_3BO_3; and (8) 100 mg $NaMoO_4$*$2H_2O$; pH is adjusted to ~3.0.

[0373] An additional exemplary minimal media includes (1) potassium phosphate K_2HPO_4, (2) Magnesium Sulfate $MgSO_4$ * $7H_2O$, (3) citric acid monohydrate $C_6H_8O_7$*H_2O, (4) ferric ammonium citrate $NH_4FeC_6H_5O_7$, (5) yeast extract (from biospringer), (6) 1000X Modified Trace Metal Solution, (7) sulfuric acid 50% w/v, (8) foamblast 882 (Emerald Performance Materials), and (9) Macro Salts Solution 3.36ml. All of the components are added together and dissolved in deionized H_2O and then heat sterilized. Following cooling to room temperature, the pH is adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Vitamin Solution and spectinomycin are added after sterilization and pH adjustment.

[0374] Any carbon source can be used to cultivate the host cells. The term "carbon source" refers to one or more carbon-containing compounds capable of being metabolized by a host cell or organism. For example, the cell medium used to cultivate the host cells can include any carbon source suitable for maintaining the viability or growing the host cells. In some

aspects, the carbon source is a carbohydrate (such as monosaccharide, disaccharide, oligosaccharide, or polysaccharides), or invert sugar (*e.g.*, enzymatically treated sucrose syrup).

[0375] In some aspects, the carbon source includes yeast extract or one or more components of yeast extract. In some aspects, the concentration of yeast extract is 0.1% (w/v), 0.09% (w/v), 0.08% (w/v), 0.07% (w/v), 0.06% (w/v), 0.05% (w/v), 0.04% (w/v), 0.03% (w/v), 0.02% (w/v), or 0.01% (w/v) yeast extract. In some aspects, the carbon source includes both yeast extract (or one or more components thereof) and another carbon source, such as glucose.

[0376] Exemplary monosaccharides include glucose and fructose; exemplary oligosaccharides include lactose and sucrose, and exemplary polysaccharides include starch and cellulose. Exemplary carbohydrates include C6 sugars (*e.g.*, fructose, mannose, galactose, or glucose) and C5 sugars (*e.g.*, xylose or arabinose).

[0377] In some aspects, the cells described herein are capable of using syngas as a source of energy and/or carbon. In some embodiments, the syngas includes at least carbon monoxide and hydrogen. In some embodiments, the syngas further additionally includes one or more of carbon dioxide, water, or nitrogen. In some embodiments, the molar ratio of hydrogen to carbon monoxide in the syngas is 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 3.0, 4.0, 5.0, or 10.0. In some embodiments, the syngas comprises 10, 20, 30, 40, 50, 60, 70, 80, or 90% by volume carbon monoxide. In some embodiments, the syngas comprises 10, 20, 30, 40, 50, 60, 70, 80, or 90% by volume hydrogen. In some embodiments, the syngas comprises 10, 20, 30, 40, 50, 60, 70, 80, or 90% by volume carbon dioxide. In some embodiments, the syngas comprises 10, 20, 30, 40, 50, 60, 70, 80, or 90% by volume water. In some embodiments, the syngas comprises 10, 20, 30, 40, 50, 60, 70, 80, or 90% by volume nitrogen.

[0378] Synthesis gas may be derived from natural or synthetic sources. The source from which the syngas is derived is referred to as a "feedstock." In some embodiments, the syngas is derived from biomass (e.g., wood, switch grass, agriculture waste, municipal waste) or carbohydrates (e.g., sugars). In other embodiments, the syngas is derived from coal, petroleum, kerogen, tar sands, oil shale, or natural gas. In other embodiments, the syngas is derived from rubber, such as from rubber tires.

[0379] Syngas can be derived from a feedstock by a variety of processes, including methane reforming, coal liquefaction, co-firing, fermentative reactions, enzymatic reactions, and biomass gasification. Biomass gasification is accomplished by subjecting biomass to partial oxidation in a reactor at temperatures above about 700 °C in the presence of less than a stoichiometric amount of oxygen. The oxygen is introduced into the bioreactor in the form of air, pure oxygen, or steam. Gasification can occur in three main steps: 1) initial heating to dry out any moisture embedded in the biomass; 2) pyrolysis, in which the biomass is heated to 300-500 °C in the absence of oxidizing agents to yield gas, tars, oils and solid char residue; and 3) gasification of solid char, tars and gas to yield the primary components of syngas. Co-firing is accomplished by gasification of a coal/biomass mixture. The composition of the syngas, such as the identity and molar ratios of the components of the syngas, can vary depending on the feedstock from which it is derived and the method by which the feedstock is converted to syngas.

[0380] Synthesis gas can contain impurities, the nature and amount of which vary according to both the feedstock and the process used in production. Fermentations may be tolerant to some impurities, but there remains the need to remove from the syngas materials such as tars and particulates that might foul the fermentor and associated equipment. It is also advisable to remove compounds that might contaminate the isoprene product such as volatile organic compounds, acid gases, methane, benzene, toluene, ethylbenzene, xylenes, H_2S, COS, CS_2, HCl, O_3, organosulfur compounds, ammonia, nitrogen oxides, nitrogen-containing organic compounds, and heavy metal vapors. Removal of impurities from syngas can be achieved by one of several means, including gas scrubbing, treatment with solid-phase adsorbents, and purification using gas-permeable membranes.

Exemplary Cell Culture Conditions

[0381] Materials and methods suitable for the maintenance and growth of the recombinant cells of the invention are described *infra*, e.g., in the Examples section. Other materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Exemplary techniques can be found in International Publication No. WO 2009/076676, U.S. Patent Publ. No. 2009/0203102, WO 2010/003007, US Publ. No. 2010/0048964, WO 2009/132220, US Publ. No. 2010/0003716, *Manual of Methods for General Bacteriology* Gerhardt *et al.*, *eds*), American Society for Microbiology, Washington, D.C.

(1994) or Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA. In some aspects, the cells are cultured in a culture medium under conditions permitting the expression of phosphoketolase polypeptide, as well as other enzymes from the upper and lower MVA pathway, including but not limited to, the *mvaE* and *mvaS* gene products, isoprene synthase, DXP pathway (e.g., DXS), IDI, or PGL polypeptides encoded by a nucleic acid inserted into the host cells.

[0382] Standard cell culture conditions can be used to culture the cells (*see*, for example, WO 2004/033646 and references cited therein). In some aspects, cells are grown and maintained at an appropriate temperature, gas mixture, and pH (such as at about 20°C to about 37°C, at about 6% to about 84% CO_2, and at a pH between about 5 to about 9). In some aspects, cells are grown at 35°C in an appropriate cell medium. In some aspects, the pH ranges for fermentation are between about pH 5.0 to about pH 9.0 (such as about pH 6.0 to about pH 8.0 or about 6.5 to about 7.0). Cells can be grown under aerobic, anoxic, or anaerobic conditions based on the requirements of the host cells. In addition, more specific cell culture conditions can be used to culture the cells. For example, in some embodiments, the recombinant cells (such as *E. coli* cells) comprise one or more heterologous nucleic acids encoding a phosphoketolase polypeptide, as well as enzymes from the upper, including but not limited to, the *mvaE* and *mvaS* gene products mvaE and mvaS polypeptides from *L. grayi, E. faecium, E. gallinarum, E. casseliflavus* and/or *E. faecalis* under the control of a strong promoter in a low to medium copy plasmid and are cultured at 34°C.

[0383] Standard culture conditions and modes of fermentation, such as batch, fed-batch, or continuous fermentation that can be used are described in International Publication No. WO 2009/076676, U.S. Patent Publ. No. 2009/0203102, WO 2010/003007, US Publ. No. 2010/0048964, WO 2009/132220, US Publ. No. 2010/0003716. Batch and Fed-Batch fermentations are common and well known in the art and examples can be found in Brock, Biotechnology: *A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc.

[0384] In some aspects, the cells are cultured under limited glucose conditions. By "limited glucose conditions" is meant that the amount of glucose that is added is less than or about 105% (such as about 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%) of the amount of glucose that is consumed by the cells. In particular aspects, the amount of glucose

that is added to the culture medium is approximately the same as the amount of glucose that is consumed by the cells during a specific period of time. In some aspects, the rate of cell growth is controlled by limiting the amount of added glucose such that the cells grow at the rate that can be supported by the amount of glucose in the cell medium. In some aspects, glucose does not accumulate during the time the cells are cultured. In various aspects, the cells are cultured under limited glucose conditions for greater than or about 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, or 70 hours. In various aspects, the cells are cultured under limited glucose conditions for greater than or about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 95, or 100% of the total length of time the cells are cultured. While not intending to be bound by any particular theory, it is believed that limited glucose conditions can allow more favorable regulation of the cells.

[0385] In some aspects, the recombinant cells are grown in batch culture. The recombinant cells can also be grown in fed-batch culture or in continuous culture. Additionally, the recombinant cells can be cultured in minimal medium, including, but not limited to, any of the minimal media described above. The minimal medium can be further supplemented with 1.0 % (w/v) glucose, or any other six carbon sugar, or less. Specifically, the minimal medium can be supplemented with 1% (w/v), 0.9% (w/v), 0.8% (w/v), 0.7% (w/v), 0.6% (w/v), 0.5% (w/v), 0.4% (w/v), 0.3% (w/v), 0.2% (w/v), or 0.1% (w/v) glucose. Additionally, the minimal medium can be supplemented 0.1% (w/v) or less yeast extract. Specifically, the minimal medium can be supplemented with 0.1% (w/v), 0.09% (w/v), 0.08% (w/v), 0.07% (w/v), 0.06% (w/v), 0.05% (w/v), 0.04% (w/v), 0.03% (w/v), 0.02% (w/v), or 0.01% (w/v) yeast extract. Alternatively, the minimal medium can be supplemented with 1% (w/v), 0.9% (w/v), 0.8% (w/v), 0.7% (w/v), 0.6% (w/v), 0.5% (w/v), 0.4% (w/v), 0.3% (w/v), 0.2% (w/v), or 0.1% (w/v) glucose and with 0.1% (w/v), 0.09% (w/v), 0.08% (w/v), 0.07% (w/v), 0.06% (w/v), 0.05% (w/v), 0.04% (w/v), 0.03% (w/v), 0.02% (w/v), or 0.01% (w/v) yeast extract.

Exemplary Purification Methods

[0386] In some aspects, any of the methods described herein further include a step of recovering the compounds produced. In some aspects, any of the methods described herein further include a step of recovering the isoprene. In some aspects, the isoprene is recovered by absorption stripping (See, *e.g.*, U.S. Publ. No. 2011/0178261). In some aspects, any of the methods described herein further include a step of recovering the heterologous polypeptide. In

some aspects, any of the methods described herein further include a step of recovering the terpenoid or carotenoid.

[0387] Suitable purification methods are described in more detail in U.S. Patent Application Publication US2010/0196977 A1.

[0388] Throughout this specification, various patents, patent applications and other types of publications (e.g., journal articles) are referenced. The disclosure of all patents, patent applications, and publications cited herein are hereby incorporated by reference in their entirety for all purposes.

[0389] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

EXAMPLES

Example 1: Cloning of the gene encoding phosphoketolase enzyme from *Bifidobacterium infantis*

[0390] Chromosomal DNA of *Bifidobacterium infantis* was obtained from ATCC (ATCC# 15697D-5, ATCC, Manassas, VA). The gene encoding phosphoketolase (PKL) enzyme was amplified using primers CMP283: 5'-ctgtatTCATGAcgagtcctgttattggcacc-3' (SEQ ID NO:30) and CMP284: 5'-ctctatGAATTCTCACTCGTTGTCGCCAGCG-3' (SEQ ID NO:31), 100 ng DNA as template and the polymerase Herculase II Fusion according to the manufacturer (Agilent, Santa Clara, CA). After purification, the 2798 bp fragment was digested with BspHI and EcoRI, and ligated with NcoI/EcoRI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) to form plasmid pCMP1090 (SEQ ID NO: 15 – Figure 6).

Example 2: Cloning of phosphoketolase enzyme from *Lactobacillus reuteri* strain F275

[0391] Chromosomal DNA of *Lactobacillus reuteri* strain F275 was obtained from ATCC (ATCC# 23272D-5, ATCC, Manassas, VA). The gene encoding phosphoketolase (PKL) enzyme was amplified using primers CMP34: 5'-taaggaggaataaacATGGCAGTAGATTACGATTCCAAG-3' (SEQ ID NO:32) and CMP335: 5'-ttctagaaagcttcgttacttaagacccttccaagtccag-3' (SEQ ID NO:33), 100 ng DNA as template and the

polymerase Herculase II Fusion according to the manufacturer (Agilent, Santa Clara, CA). After purification, the 2442 bp fragment was assembled into NcoI/EcoRI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1029 (SEQ ID NO:16 – Figure 7).

Example 3: Construction of strains CMP451, CMP674, CMP1015 and CMP1047 (BL21 GI1.2gltA ldhA)

[0392] The promoter in front of the citrate synthase gene (*gltA)* in *E. coli* strain BL21 (Novagen) was previously replaced by a constitutive low expression promoter, namely GI1.2 (US patent 7,371,558). Two wild-type promoters have been described for *gltA* (Wilde, R, and J. Guest. 1986. *J. Gen. Microbiol.* 132:3239-3251). The synthetic promoter was inserted just after the -35 region of the distal promoter. A PCR product was obtained using primers UpgltACm-F (5'-TATTTAATTTTTAATCATCTAATTTGACAATCATTCAACAAAGTTGTTACAATTAACCCTCACTAAAGGGCGG-3' (SEQ ID NO:34)) and DngltA1.xgiCm-R (5'-TCAACAGCTGTATCCCCGTTGAGGGTGAGTTTTGCTTTTGTATCAGCCATATATTCCACCAGCTATTTGTTAGTGAATAAAAGTGGTTGAATTATTTGCTCAGGATGTGGCATHGTCAAGGGCTAATACGACTCACTATAGGGCTCG-3' (SEQ ID NO:35)), and plasmid FRT-gb2-Cm-FRT from Gene Bridges (Heidelberg, Germany) as a template. The PCR product was purified and used in a lambda red-mediated recombination as described by the manufacturer (Gene Bridges, Heidelberg, Germany). Several colonies were selected for further characterization. The promoter region was PCR-amplified using primers gltAPromSeqF: 5'-GGCAGTATAGGCTGTTCACAAAATC-3' (SEQ ID NO:36) and gltApromSeqR: 5'-CTTGACCCAGCGTGCCTTTCAGC-3' (SEQ ID NO:37) and, as a template, DNA extracted by resuspending a colony in 30 uL H2O, heating at 95 C for 4 min, spinning down, and using 2 uL of that material as a template in a 50 uL reaction. After observing the sequencing results of the PCR products obtained, a colony harboring the GI1.2 promoter (US patent 7,371,558) was named CMP141.

[0393] Strain MD09-313 was built by transducing CMP258 (see U.S. Patent Application No. 12/978,324) with a P1 lysate from strain MCM521 (see U.S. Patent Application No. 12/978,324) and selecting for colonies on Luria-Bertani plates containing 20 ug/ml

kanamycin. P1 lysates are prepared according to the method described in Ausubel, et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. The kanamycin marker was removed using the protocol recommended by the manufacturer (Gene Bridges, Heidelberg, Germany) to form strain MD09-314.

[0394] A P1 lysate was made from strain CMP141 was used to transduce strain MD09-314, to form CMP440. The chloramphenicol marker was removed using the protocol recommended by the manufacturer (Gene Bridges, Heidelberg, Germany) to form strains CMP451.

[0395] A DNA fragment containing a chloramphenicol marker flanked by DNA homologous to the upstream and downstream regions of the λ attachment site attB was amplified by PCR using plasmid pKD3 (Datsenko, K., and Wanner, B. 2000. *PNAS* 97:6640-6645) as a template, and primers CMP171 (5'-AAAATTTTCATTCTGTGACAGAGAAAAAGTAGCCGAAGATGACGGTTTGTCACATG GAGTTGGCAGGATGTTTGATTACATGGGAATTAGCCATGGTCC-3' (SEQ ID NO:38)) and CMP172 (5'-GACCAGCCGCGTAACCTGGCAAAATCGGTTACGGTTGAGTAATAAATGGATGCCCT GCGTAAG CGG GGCATT TTTCTTGGTGTAGGCTGGAGCTGCTTCG-3' (SEQ ID NO:39)). The PCR product obtained was used in a recombineering reaction in BL21 (Novagen) as recommended by the manufacturer (Gene Bridges, Heidelberg, Germany) to integrate the PCR product at the λ attachment site attB. Strain CMP646 was thereby generated, selected on LB + 5 ug/ml chloramphenicol. A P1 lysate of CMP646 was made and was used in a transduction reaction on strain CMP451, thereby removing the lower mevalonate pathway genes (encoding mevalonate kinase, phosphomevalonate kinase, diphosphomevalonate decarboxylase, and isopentenyl diphosphate isomerase) from the chromosome of that strain. The transduction reaction was plated on LB + chloramphenicol 5 ug/ml and one colony for each transduction was picked and named CMP674.

Table 2: Description of *E. coli* strains

Strain	Description	Parent

CMP141	BL21 Cm-GI1.2 gltA	BL21
CMP258	BL21 pgl+	BL21
CMP374	BL21 pgl+ PL.2-mKKDyI ldhA::Kan	MD09-314
CMP440	BL21 pgl+ PL.2 mKKDyI Cm-GI1.2 gltA	MD09-314
CMP451	BL21 pgl+ PL.2 mKKDyI GI1.2 gltA	CMP440
MCM521	BL21 neo-PL.2-mKKDyI	U.S. Patent App. No: 12/978,324
CMP646	BL21 attB:Cm (to restore LowerP)	BL21 (Novagen)
CMP674	BL21 pgl+ GI 1.2 gltA attB::Cm	CMP451
CMP1015	BL21 pgl+ GI 1.2 gltA ldhA::Kan attB::Cm,	CMP674
CMP1036	BL21 pgl+ GI 1.2 gltA attB::Cm, pTrcHis2B	CMP674
CMP1038	BL21 pgl+ GI 1.2 gltA attB::Cm, pTrcPKL *Bifido*	CMP674
CMP1040	BL21 pgl+ GI 1.2 gltA attB::Cm, pTrcPKL *L.reuteri*	CMP674
CMP1047	BL21 pgl+ GI 1.2 gltA ldhA	CMP1015
CMP1053	BL21 pgl+ GI 1.2 gltA ldhA, pTrcHis2B, pCLPtrcUpper*E.faecalis*	CMP1047
CMP1055	BL21 pgl+ GI 1.2 gltA ldhA, pTrcPKL *Bifido*, pCLPtrcUpper*E.faecalis*	CMP1047

CMP1057	BL21 pgl+ GI 1.2 gltA ldhA, pTrcPKL *L.reuteri*, pCLPtrcUpper*E.faecalis*	CMP1047

[0396] A DNA fragment containing the ldhA gene interrupted by a kanamycin marker was amplified by PCR using strain JW 1375 from the Keio collection (Baba et al. 2006. *Mol. Syst. Biol.* 2: 2006.0008) as a template, and primers ldhAseqR (5'-GGCTTACCGTTTACGCTTTCCAGC-3' (SEQ ID NO:40)) and ldhAseqF2 (5'-CTAATGCAATACGTGTCCCGAGC-3' (SEQ ID NO:41)). The PCR product obtained was used in a recombineering reaction as recommended by the manufacturer (Gene Bridges, Heidelberg, Germany) to integrate the PCR product at the ldhA locus in strain CMP674. That strain was named CMP1015. The chloramphenicol and kanamycin markers were looped out simultaneously by electroporating pCP20 (Datsenko and Wanner. 2000. *PNAS* 97:6640-6645) in the strain, selecting two colonies on LB + 50 ug/ml carbenicillin at 30°C, then restreaking those colonies on an LB plate at 42°C. A Cm^S and Kan^S colony was selected from those plates and named CMP1047.

Example 4: Construction of strains CMP1036, 1038 and 1040

[0397] Strain CMP674 was electroporated in the presence of plasmids pTrcHis2B, pCMP1090 (PKL from *Bifidobacterium infantis*) and pCMP1029 (PKL from *Lactobacillus reuteri*). Colonies were isolated on LB + carbenicillin 50 ug/mL. One colony of each transformation was picked and was named CMP1036, CMP1038 and CMP1040 respectively.

Example 5: Measurement of acetyl phosphate in strains CMP1036, 1038 and 1040

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0398] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-

sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotic are added after pH adjustment and sterilization.

1000X Trace Metal Solution (per liter fermentation media)

[0399] Citric Acid*H_2O 40g, $MnSO_4$*H_2O 30g, NaCl 10g, $FeSO_4$*$7H_2O$ 1g, $CoCl_2$*$6H_2O$ 1g, $ZnSO_4$*$7H_2O$ 1g, $CuSO_4$*$5H_2O$ 100mg, H_3BO_3 100mg, $NaMoO_4$*$2H_2O$ 100mg. Each component is dissolved one at a time in diH_2O. The pH is adjusted to 3.0 with HCl/NaOH, and then the solution is brought to volume and filter-sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

[0400] Cells were grown overnight in Luria-Bertani broth + antibiotics. The day after, they were diluted to an OD600 of 0.05 in 20 mL TM3 medium containing 50 ug/mL carbenicillin (in a 250-mL baffled Erlenmeyer flask), and incubated at 34°C and 200 rpm. After 2h of growth, OD600 was measured and 200 uM IPTG was added. After 3.5 more hours, 1.5 ml sample was centrifuged, the supernatant was discarded and the pellet was resuspended in 100 uL dry-ice cold methanol.

(iii) Intracellular acetyl-phosphate determination.

[0401] To extract acetyl-phosphate, 1.5 mL of E.coli cells grown to OD 0.57-2.26 was spun down by centrifugation and 100 µL of dry-ice cold methanol was added to the pellets. Methanol-quenched samples were stored at -20°C for several days. Further sample processing included gentle cell re-suspension, 5-min centrifugation at -9°C and aspiration of the supernatant into clean vials. The pellet was re-extracted twice with 75 µL of water containing 2% acetic acid. After each extraction, cell debris were pelleted by centrifugation at -9°C, the supernatants from all three extractions were pooled together and spiked with 1 µL of tributylamine. Mass spectrometric analysis of acetyl phosphate by LCMS was carried out using a Thermo Finnigan TSQ system (Thermo Electron Corporation, San Jose, CA). The system control, data acquisition, and mass spectral data evaluation were performed using XCalibur and LCQuan software (Thermo Electron Corp). A mobile phase gradient was applied to a Synergi MAX-RP 5µM HPLC column (150 x 2 mm, Phenomenex) at a flow rate of 0.4 mL/min. The applied gradient profile was 99% A and 1% B at t=0-1 min; 80% A and 20% B at t=11 min; 75% B and 25% C at

t=12-14 min; 99% A and 1% B at t=15-16 min, where solvent A was 15mM tributylamine/10 mM acetic acid in water, solvent B was methanol, and solvent C was water. Mass detection of acetyl phosphate was carried out using electrospray ionization (ESI-MS/MS) in the negative mode (ESI spray voltage of 2.5-3.0 kV, ion transfer tube temperature 390°C) with m/z value for the precursor ion of 138.9. Concentration of acetyl phosphate was determined based on the integrated intensity of peak generated by PO_3^- product ion (m/z =79.0, collision energy 20 V, collision gas pressure 1.7 mTorr, R_t=13.2 min). Calibration curve obtained by injection of acetyl phosphate standard (Sigma-Aldrich) was used to calculate concentration of the metabolite in cell extracts. Intracellular concentration of acetyl phosphate was determined based on the assumption that in 1 mL of the culture at OD=200 the integrated volume of all cells is 50 Ml (Figure 8).

(iv) Results

[0402] Strains expressing phosphoketolase had higher intracellular concentrations of acetyl phosphate (CMP1040 and CMP1038) than the control strain not expressing phophoketolase (Figure 8).

Example 6: Construction of strains CMP1053, 1055 and 1057

[0403] Plasmids pTrcHis2B (Invitrogen, Carlsbad, CA), pCMP1090 (PKL from *Bifidobacterium infantis*) or pCMP1029 (PKL from *Lactobacillus reuteri*) were used to transform CMP1047 together with plasmid pMCM82 (expression vector MCM82 (U.S. Patent Application Publication No. US2010/0196977). Host CMP1047 was grown to mid-log in LB at 34C and prepared for electroporation by washing 2x in one culture volume of iced ddH2O and resuspended in one tenth culture volume of the same. 100uL of cell suspension was combined with 1uL of each plasmid DNA, moved to a 2mm electroporation cuvette, electroporated at 25uFD, 200 Ohms, 2.5kV, and immediately quenched with 1 mL LB. Cells were recovered shaking at 34C for 1hr and then transformants selected overnight on LB plates with 50ug /mL spectinomycin + 50 ug/mL carbenicillin at 34C. One colony for each transformation was picked and named CMP1053, 1055 and 1057 respectively.

[0404] In other embodiments, any of the resultant strains described above can be further engineered to modulate the activity of the Entner-Doudoroff pathway. The gene coding for phosphogluconate dehydratase or aldolase can be attenuated or deleted. In other embodiments, any of the resultant strains described above may also be engineered to decrease or

remove the activity of acetate kinase or citrate synthase. In other embodiments, any of the strains the resultant strain may also be engineered to decrease or remove the activity of phosphofructokinase. In other embodiments, any of the resultant strains described above may also be engineered to modulate the activity of glyceraldehydes-3-phosphate dehydrogenase. The activity of glyceraldehydes 3-phosphate dehydrogenase can be modulated by decreasing its activity. In other embodiments, the enzymes from the non-oxidative branch of the pentose phosphate pathway, such as transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can be overexpressed.

Example 7: Production of mevalonate by strains CMP1053, 1055 and 1057

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0405] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotics are added after pH adjustment and sterilization.

1000X Trace Metal Solution (per liter fermentation media)

[0406] Citric Acid$*H_2O$ 40g, $MnSO_4*H_2O$ 30g, NaCl 10g, $FeSO_4*7H_2O$ 1g, $CoCl_2*6H_2O$ 1g, $ZnSO_4*7H_2O$ 1g, $CuSO_4*5H_2O$ 100mg, H_3BO_3 100mg, $NaMoO_4*2H_2O$ 100mg. Each component is dissolved one at a time in diH_2O. The pH is adjusted to 3.0 with HCl/NaOH, and then the solution is brought to volume and filter-sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

[0407] Cells are grown overnight in Luria-Bertani broth + antibiotics. The day after, they are diluted to an OD600 of 0.05 in 20 mL TM3 medium containing 50 ug/ml of spectinomycin and 50 ug/mL carbenicillin (in a 250-mL baffled Erlenmeyer flask), and incubated at 34°C and 200 rpm. After 2h of growth, OD600 is measured and 200 uM IPTG is

added. Samples are taken regularly during the course of the fermentation. At each timepoint, OD600 is measured. After 24 h, mevalonate is analyzed by HPLC. HPLC analysis was performed in the following way: 54 uL of 10% (w/v) H2SO4 was added to 300 uL of broth and the mixture was incubated on ice for 5 minutes. Next, the sample was centrifuged at 14,000×g for 5 minutes and the supernatant collected for HPLC analysis run under the following conditions: (1) BioRad - Aminex HPX-87H Ion Exclusion Column (300 mm x 7.8 mm)(Catalog # 125-0140)(BioRad, Hercules, California); (2) column temperature = 50°C; (3) BioRad - Microguard Cation H guard colum refill (30 mm x 4.6 mm)(Catalog # 125-0129)(BioRad); (4) running buffer = 0.01N H2SO4; (5) running buffer flow rate = 0.6 ml / min; (6) approximate running pressure = ~950 psi; (7) injection volume = 20 microliters; (8) runtime = 26 minutes.

(iii) *Results*:

[0408] Strains expressing phosphoketolase grew slower than the control strain (Figure 9). CMP1057 (expressing the *L.* reuteri phosphoketolase gene) produced more mevalonate than the strains containing the control empty plasmid or the phosphoketolase from *B. infantis* (Figure 10).

Example 8: Construction of strains producing isoprene and expressing phosphoketolase

[0409] A lower mevalonate pathway is introduced by transduction into CMP674 using a lysate from MCM521 (see Table 2). The kanamycin marker is looped out according to the manufacturer (Gene Bridges, Heidelberg, Germany). The lower pathway from MCM521 can be modified by changing the promoter upstream of the operon by modifying the rbs in front of each gene via the use of alternative genes. An expression plasmid expressing lacI, isoprene synthase and *M. mazei* mevalonate kinase, plasmid pMCM82 (expression vector MCM82 (U.S. Patent Application Publication No. US2010/0196977)) and plasmid pCMP1090 (PKL from *Bifidobacterium infantis*), pCMP1029 (PKL from *Lactobacillus reuteri*) or pTrcHis2B are electroporated (in two steps) into CMP1047. Colonies are selected on LB+ spectinomycin 50 ug/mL + carbenicillin 50 ug/mL + chloramphenicol 25 ug/mL.

[0410] In other embodiments, any of the resultant strains described above can be further engineered to modulate the activity of the Entner-Doudoroff pathway. The gene coding for phosphogluconate dehydratase or aldolase can be attenuated or deleted. In other

embodiments, any of the resultant strains described above may also be engineered to decrease or remove the activity of acetate kinase or citrate synthase. In other embodiments, any of the strains the resultant strain may also be engineered to decrease or remove the activity of phosphofructokinase. In other embodiments, any of the resultant strains described above may also be engineered to modulate the activity of glyceraldehyde-3-phosphate dehydrogenase. The activity of glyceraldehyde-3-phosphate dehydrogenase can be modulated by decreasing its activity. In other embodiments, the enzymes from the non-oxidative branch of the pentose phosphate pathway, such as transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can be overexpressed.

Example 9: Production of isoprene by strains harboring a plasmid expressing the upper mevalonate pathway , a plasmid expressing lacI, isoprene synthase and mevalonate kinase, and a plasmid expressing phosphoketolase in comparison to cells harboring a plasmid expressing the upper mevalonate pathway , a plasmid expressing lacI, isoprene synthase and mevalonate kinase, and an empty plasmid (pTrcHis2B)

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0411] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotics are added after pH adjustment and sterilization.

1000X Trace Metal Solution (per liter fermentation media)

[0412] Citric Acid$*H_2O$ 40g, $MnSO_4*H_2O$ 30g, NaCl 10g, $FeSO_4*7H_2O$ 1g, $CoCl_2*6H_2O$ 1g, $ZnSO_4*7H_2O$ 1g, $CuSO_4*5H_2O$ 100mg, H_3BO_3 100mg, $NaMoO_4*2H_2O$ 100mg. Each component is dissolved one at a time in diH_2O. The pH is adjusted to 3.0 with HCl/NaOH, and then the solution is brought to volume and filter-sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

[0413] Cells are grown overnight in Luria-Bertani broth + antibiotics. The day after, they are diluted to an OD600 of 0.1 in 20 mL TM3 medium containing 50 ug/ml of spectinomycin, 25 ug/mL chloramphenicol and 50 ug/mL carbenicillin (in a 250-mL baffled Erlenmeyer flask), and incubated at 34°C and 200 rpm. After 2h of growth, OD600 is measured and 200 uM IPTG is added. Samples are taken regularly during the course of the fermentation. At each timepoint, OD600 is measured. Also, off-gas analysis of isoprene is performed using a gas chromatograph-mass spectrometer (GC-MS) (Agilent) headspace assay. One hundred microliters of whole broth are placed in a sealed GC vial and incubated at 34°C and 200 rpm for a fixed time of 30 minutes. Following a heat kill step, consisting of incubation at 70°C for 7 minutes, the sample is loaded on the GC. The reported specific productivity is the amount of isoprene in ug/L read by the GC divided by the incubation time (30 min) and the measured OD600.

(iii) *Results*:

[0414] The strains expressing phosphoketolase grow more slowly than the control strain which does not express phosphoketolase. Strains expressing the phosphoketolase polypeptide display enhanced production of isoprene as compared to the the strains containing the control empty plasmid (i.e., the strain that does not express phosphoketolase) due to the observance of increased specific productivity, yield, CPI and/or titer of isoprene in the strains expressing the phosphoketolase polypeptide.

Example 10: Construction of strains expressing phosphoketolase and producing amorphadiene or farnesene

[0415] A lower mevalonate pathway is introduced by transduction into CMP674 using a lysate from MCM521 (see Table 2). The kanamycin marker is looped out according to the manufacturer (Gene Bridges, Heidelberg, Germany). The lower pathway from MCM521 can be modified by changing the promoter upstream of the operon by modifying the rbs in front of each gene via the use of alternative genes. Farnesyl diphosphate synthase (*ispA*) is overexpressed, either by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid.

[0416] The expression plasmid expressing lacI, isoprene synthase and *M. mazei* mevalonate kinase from example 8 is modified to replace the gene coding for isoprene synthase by a codon-optimized gene coding for farnesene synthase or amorphadiene synthase. The following expression plasmids are electroporated (in two steps) into competent host cells: (i) the plasmid having lacI, farnesene synthase or amorphadiene synthase, and *M. mazei* mevalonate kinase, (ii) pMCM82 (expression vector MCM82 (U.S. Patent Application Publication No. US2010/0196977) and (iii) pCMP1090 (PKL from *Bifidobacterium infantis*), or pCMP1029 (PKL from *Lactobacillus reuteri*) or pTrcHis2B. Colonies are selected on LB+ spectinomycin 50 ug/mL + carbenicillin 50 ug/mL + chloramphenicol 25 ug/mL.

[0417] In other embodiments, any of the resultant strains described above can be further engineered to modulate the activity of the Entner-Doudoroff pathway. The gene coding for phosphogluconate dehydratase or aldolase can be attenuated or deleted. In other embodiments, any of the resultant strains described above may also be engineered to decrease or remove the activity of acetate kinase or citrate synthase. In other embodiments, any of the strains the resultant strain may also be engineered to decrease or remove the activity of phosphofructokinase. In other embodiments, any of the resultant strains described above may also be engineered to modulate the activity of glyceraldehyde-3-phosphate dehydrogenase. The activity of glyceraldehyde-3-phosphate dehydrogenase can be modulated by decreasing its activity. In other embodiments, the enzymes from the non-oxidative branch of the pentose phosphate pathway, such as transketolase, transaldolase, ribulose-5-phosphate-epimerase and (or) ribose-5-phosphate epimerase can be overexpressed.

Example 11: Production of amorphadiene or farnesene in strains containing a plasmid expressing phosphoketolase in comparison with the control strain

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0418] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is then filter-

sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotics are added after sterilization and pH adjustment.

1000X Trace Metal Solution (per liter fermentation media):

[0419] Citric Acid*H_2O 40g, $MnSO_4$*H_2O 30g, NaCl 10g, $FeSO_4$*$7H_2O$ 1g, $CoCl_2$*$6H_2O$ 1g, $ZnSO_4$*$7H_2O$ 1g, $CuSO_4$*$5H_2O$ 100mg, H_3BO_3 100mg, $NaMoO_4$*$2H_2O$ 100mg. Each component is dissolved one at a time in diH_2O. The pH is adjusted to 3.0 with HCl/NaOH, and then the solution is brought to volume and filter-sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

[0420] Cells are grown overnight in Luria-Bertani broth + antibiotics. The day after, they are diluted to an OD600 of 0.05 in 20 mL TM3 medium containing 50 ug/ml of spectinomycin, 25 ug/mL chloramphenicol and 50 ug/mL carbenicillin (in a 250-mL baffled Erlenmeyer flask), and incubated at 34°C and 200 rpm. Prior to inoculation, an overlay of 20% (v/v) dodecane (Sigma-Aldrich) is added to each culture flask to trap the volatile sesquiterpene product as described previously (Newman et. al., 2006).

[0421] After 2h of growth, OD600 is measured and 0.05-0.40 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) is added. Samples are taken regularly during the course of the fermentation. At each timepoint, OD600 is measured. Also, amorphadiene or farnesene concentration in the organic layer is assayed by diluting the dodecane overlay into ethyl acetate. Dodecane/ethyl acetate extracts are analyzed by GC–MS methods as previously described (Martin et. al., *Nat.* Biotechnol. 2003, 21:96–802) by monitoring the molecular ion (204 m/z) and the 189 m/z fragment ion for amorphadiene or the molecular ion (204 m/z) for farnesene. Amorphadiene or farnesene samples of known concentration are injected to produce standard curves for amorphadiene or farnesene, respectively. The amount of amorphadiene or farnesene in samples is calculated using the amorphadiene or farnesene standard curves, respectively.

(iii) *Results*

[0422] The strains expressing the phosphoketolase polypeptide are compared to the strains containing an empty plasmid (i.e., lacking the phospoketolase polypeptide) with the same

backbone. The strains expressing the phosphoketolase polypeptide display enhanced production of amorphadiene or farnesene as compared to the the strains containing containing the control empty plasmid (i.e., the strain that does not express phosphoketolase) due to the observance of increased specific productivity, yield, CPI and/or titer of amorphadiene or farnesene in the strains expressing the phosphoketolase polypeptide.

(iv) *References*

[0423] Newman, J.D., Marshal, J.L., Chang, M.C.Y., Nowroozi, F., Paradise,E.M., Pitera, D.J., Newman, K.L., Keasling, J.D., 2006. High-level production of amorpha-4,11-diene in a two-phase partitioning bioreactor of metabolically engineered *E. coli. Biotechnol. Bioeng.*95, 684–691.

[0424] Martin, V.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D.,2003. Engineering a mevalonate pathway in *E. coli* for production of terpenoids. *Nat. Biotechnol.* 21, 796–802.

Example 12: Production of Mevalonate (MVA) in recombinant host cells expressing Phosphoketolase at 15-L Scale

[0425] Mevalonate production was evaluated in *E. coli* expressing a heterologous gene encoding a phosphoketolase polypeptide as well as genes from the mevalonate pathway and grown in fed-batch culture at the 15-L scale.

[0426] An MVA producing strain SHG0863 (CMP1053-HMB GI1.2gltA attB ldhA, pTrcHis2B, pCLPtrcUpperEfaecalis) was run in a standard MVA production process. The performance metrics (MVA productivity and MVA yield on glucose) are compared here to an experimental strain SHG0864 (CMP1057-HMB GI1.2gltA attB ldhA, pTrcPKL_Lreuteri, pCLPtrcUpperEfaecalis) that was run under the same conditions to determine yield improvement attributable to the expression of the phosphoketolase polypeptide.

Methods:

Medium Recipe (per liter fermentation medium):

[0427] K2HPO4 7.5 g, MgSO4 * 7H2O 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, yeast extract 0.5 g, 50% sulphuric acid 1.6 mL, 1000X Modified Trace Metal Solution 1 ml. All of the components were added together and dissolved in Di H2O. This

solution was heat sterilized (123°C for 20 minutes). The pH was adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Glucose 10 g, Vitamin Solution 8 mL, and antibiotics were added after sterilization and pH adjustment.

1000X Modified Trace Metal Solution (per liter):

[0428] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

Vitamin Solution (per liter):

[0429] Thiamine hydrochloride 1.0 g, D-(+)-biotin 1.0 g, nicotinic acid 1.0 g, pyridoxine hydrochloride 4.0 g. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with 0.22 micron filter.

Feed solution (per kilogram):

[0430] Glucose 0.590 kg, Di H2O 0.393 kg, K2HPO4 7.4 g, and 100% Foamblast882 8.9 g. All components were mixed together and autoclaved.

Macro Salt Solution (per liter):

[0431] MgSO4 * 7H2O 296 g, citric acid monohydrate 296 g, ferric ammonium citrate 49.6 g. All components were dissolved in water, q.s. to volume and filter sterilized with 0.22 micron filter. Add 16.8mls directly the tank media before sterilization, with no further addition.

[0432] This experiment was carried out to monitor mevalonate formation from glucose at the desired fermentation pH 7.0 and temperature 34°C. A frozen vial of the *E. coli* strain was thawed and inoculated into a flask with tryptone-yeast extract medium and the appropriate antibiotics. After the inoculum grew to optical density 1.0, measured at 550 nm (OD_{550}), 500 mL was used to inoculate a 15-L bioreactor and bring the initial tank volume to 5 L.

[0433] The batched media had glucose batched in at 9.7 g/L. Induction was achieved by adding isopropyl-beta-D-1-thiogalactopyranoside (IPTG). A shot of IPTG was added to the tank to bring the concentration to 250 uM when the cells were at an OD_{550} of 6. A second shot of IPTG was added to the tank to bring the concentration to 500 uM when the cells were at an OD_{550} of 100. Once the glucose was consumed by the culture, as signaled by a rise in pH, the glucose feed solution was fed to meet metabolic demands at rates less than or equal to 10 g/min. The fermentation was run long enough to determine the maximum mevalonate mass yield on glucose, a total of 48 hr elapsed fermentation time.

Analysis:

[0434] The mevalonate concentration in the fermentor broth was determined in broth samples taken at 4 hour intervals by an HPLC analysis. Mevalonate concentration in broth samples was determined by comparison of the refractive index response versus a previously generated calibration curve.

HPLC INFORMATION

System: Waters Alliance 2695
Column: BioRad - Aminex HPX-87H Ion Exclusion Column 300mm x 7.8mm Catalog # 125-0140
Column Temperature: 50C
Guard column: BioRad - Microguard Cation H refill 30mm x 4.6mm Catalog # 125-0129
Running buffer: 0.01N H_2SO_4
Running buffer flow rate: 0.6 ml / min
Approximate running pressure: ~1100-1200 psi
Injection volume: 20 microliters
Detector: Refractive Index (Knauer K-2301)
Runtime: 26 minutes

Results:

[0435] The fermentation with the phosphoketolase expressing strain (CMP1057) had higher a mevalonate yield on glucose than the empty plasmid control strain (CMP1053). See Figures 11-13 and Table 3 below. Additionally, the fermentation broth with the phosphoketolase expressing strain (CMP1057) had lower acetate accumulation in the fermentation broth as compared to the empty plasmid control strain (CMP1053). See Figure 14.

Table 3. MVA Productivity Metrics

Strain description / Run Number	EFT (hrs)	Titer (g/L)	Volumetric Productivity (g/L/hr)	Overall % Yield of MVA on glucose (g/g)	CPI (gMVA/gDCW)
SHG0863 CMP1053 Control strain 20111003	48	72.8	1.52	25.5%	2.40
SHG0864 CMP1057 Phosphoketolase strain 20111004	48	109.9	2.29	28.2%	2.94

Example 13-Production of Isoprene by *Saccharomyces cerevisiae* expressing phosphoketolase

[0436] Variations of the genetic constructs described above for expression of isoprene synthase, the upper MVA pathway, the lower MVA pathway and a phosphoketolase are prepared using a yeast expression system from Life Technologies (including competent, wild-type *S. cerevisiae* (INVSc1 (catalog number C810-00)) and the plasmid pYES2/CT (catalog number V8251-20).

[0437] The genes encoding the isoprene synthase, the upper MVA pathway, the lower MVA pathway and a phosphoketolase are first sub-cloned into the pYES2/CT vectors. The plasmids are then propagated in *E. coli* cells. The *S. cerevisiae* (INVSc1) are then transformed with the purified plasmid DNA. Yeast strains harboring the plasmid are selected for and maintained on SC Minimal Medium with 2% glucose supplemented with the indicated selective marker. Isolated colonies harboring the plasmid are chosen for further experimentation.

[0438] The specific productivity of isoprene from the engineered yeast strains is determined. To induce expression of the genes encoded by the plasmid, cultures are grown overnight in liquid SC Minimal Medium supplemented with the selective marker. The cultures are then diluted to an OD_{600} of approximately 0.2 and grown for 2-3 hours. A 100µL sample of the broth is incubated in a 2mL headspace vial at 34°C for 30 minutes, followed by heat kill at 70°C for 12 minutes. Levels of isoprene in the headspace are determined, for example, by flame ionization detector coupled to a gas chromatograph (Model G1562A, Agilent Technologies) (Mergen et al., *LC GC North America*, 28(7):540-543, 2010).

Example 14: Cloning of phosphoketolase enzyme from various diverse bacteria

[0439] Chromosomal DNA of strain ATCC15697, *Bifidobacterium longum* subsp. *infantis* was obtained from ATCC (Manassas, VA). The gene encoding *B. longhum* PKL (SEQ ID NO:3) was amplified by polymerase chain reaction (PCR) from the chromosomal DNA using primers CMP283: 5'-ctgtatTCATGAcgagtcctgttattggcacc-3' (SEQ ID NO:42) and CMP284: 5'-ctctatGAATTCTCACTCGTTGTCGCCAGCG-3' (SEQ ID NO:43), and the polymerase Herculase according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). The PCR product was digested with EcoRI and BspHI restriction enzymes before purification. After purification, the aproximately 2500 bp fragment was assembled into EcoRI/NcoI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1090 (SEQ ID NO:15, Figure 6).

[0440] Chromosomal DNA of *Lactobacillus reuteri* strain F275 was obtained from ATCC (ATCC# 23272D-5, ATCC, Manassas, VA). The gene encoding *Lactobacillus reuteri* PKL (SEQ ID NO:1) was amplified using primers CMP34: 5'-taaggaggaataaacATGGCAGTAGATTACGATTCCAAG-3' (SEQ ID NO:44) and CMP335: 5'-ttctagaaagcttcgttacttaagacccttccaagtccag-3' (SEQ ID NO:45), 100 ng DNA as template and the polymerase Herculase II Fusion according to the manufacturer (Agilent, Santa Clara, CA). After purification, the 2442 bp fragment was assembled into NcoI/EcoRI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1029 (SEQ ID NO:16, Figure 7).

[0441] The amino acid sequence of *Enterococcus gallinarum* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in *E. coli*. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *E. gallinarum* PKL gene (SEQ ID NO:17) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1321.

[0442] Chromosomal DNA of strain ATCC27893, *Nostoc punctiforme* was obtained from ATCC (Manassas, VA). The gene encoding *N. punctiforme* PKL (SEQ ID NO:18) was amplified by polymerase chain reaction (PCR) from the chromosomal DNA using primers

NostocpTrcHis2BF: 5'-taaggaggaataaaccatgacattagccagtcctctacaaac-3' (SEQ ID NO:46) and NostocpTrcHis2BR: 5'-TTCTAGAAAGCTTCGTTAATAGGGCCACTTCCAGTCACG-3' (SEQ ID NO:47), and the polymerase Herculase according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). The PCR product was digested with EcoRI and BspHI restriction enzymes before purification. After purification, the aproximately 2500 bp fragment was assembled into EcoRI/NcoI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1305.

[0443] Chromosomal DNA of strain ATCC BAA-98, *Rhodopseudomonas palustris* was obtained from ATCC (Manassas, VA). The gene encoding *R. palustris* PKL (SEQ ID NO:19) was amplified by polymerase chain reaction (PCR) from the chromosomal DNA using primers RpalpTrcHis2BF: 5'-taaggaggaataaaccatgtccgacgtgttgtccaacgatc-3' (SEQ ID NO:48) and RpalpTrcHis2BR: 5'TTCTAGAAAGCTTCGTCAGGCCGACCAGCGCCAG-3' (SEQ ID NO:49), and the polymerase Herculase according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). The PCR product was digested with EcoRI and BspHI restriction enzymes before purification. After purification, the aproximately 2500 bp fragment was assembled into EcoRI/NcoI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1306.

[0444] The amino acid sequence of *Pantoea* sp. PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *Pantoea* sp. PKL gene (SEQ ID NO:20) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1324.

[0445] The amino acid sequence of *Mucilaginibacter paludis* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *M. paludis* PKL gene (SEQ ID NO:21) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1323.

[0446] The amino acid sequence of *Thermobifida fusca* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *T. fusca* PKL gene (SEQ ID NO:22) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1326.

[0447] The amino acid sequence of *Bfidobacterium breve* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *B. breve* PKL gene (SEQ ID NO:23) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1322.

[0448] The amino acid sequence of *Rahnella aquatilis* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *R. aquatilis* PKL gene (SEQ ID NO:24) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1325.

[0449] The amino acid sequence of *Bifidobacterium animalis* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *B. animalis* PKL gene (SEQ ID NO:25) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1320.

[0450] The amino acid sequence of *Gardnerella vaginalis* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E.

coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *G. vaginalis* PKL gene (SEQ ID NO:26) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1309.

[0451] The amino acid sequence of *Streptomyces avermitilis* PKL was obtained from GeneBank and was processed in GeneArt optimization software for optimized expression in E. coli. Two base pairs were added in front of the PKL gene to form a NcoI site and a SacI site was inserted just after the stop codon. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The *S. avermitilis* PKL gene (SEQ ID NO:27) was then subcloned into a BspHI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pEWL1362.

[0452] Chromosomal DNA of strain ATCC BAA-98, *Clostridium acetobutylicum* was obtained from ATCC (Manassas, VA). The gene encoding *Clostridium acetobutylicum* PKL (SEQ ID NO:28) was amplified by polymerase chain reaction (PCR) from the chromosomal DNA using primers CacetpTrcHisBF: 5'-taaggaggaataaaccatgcaaagtataataggaaaacataaggatgaagg-3' (SEQ ID NO:50) and CacetpTrcHisBR: 5'-ttctagaaagcttcgttatacatgccactgccaattagttatttc-3' (SEQ ID NO:51), and the polymerase Herculase according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). The PCR product was digested with EcoRI and BspHI restriction enzymes before purification. After purification, the aproximately 2500 bp fragment was assembled into EcoRI/NcoI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1364.

[0453] The amino acid sequence of Lactobacillus paraplantarum PKL was obtained from GeneBank and Jeong et al., (J. Microbiol. Biotechnol. 2007, 17:822-829), and was processed in GeneArt optimization software for optimized expression in E. coli. The synthesized PKL gene was cloned into GeneArt kanamycin-resistant cloning plasmid. The L. paraplantarum PKL gene (SEQ ID NO:29) was then amplified using primers SML_NcoI_PhosphokLplantF (taaggaggaataaacatgaccaccgattatagcagtcc) and v2SML_EcoRI_PhosphokLplantR (ttctagaaagcttcgTTA TTT CAG ACC TTT CCA CTG CC), and Herculase (Life Technologies, Carlsbad, CA) as polymerase according to the manufacter's

protocol. The PCRs obtained was then assembled with an EcoRI/NcoI-digested pTrcHis2B plasmid (Life Technologies, Carlsbad, CA) using the GeneArt® seamless cloning and assembly kit (Life Technologies, Carlsbad, CA) to form plasmid pCMP1184.

Example 15: Construction of strains CMP1183, CMP1328, CMP 1366, CMP1182, CMP1308, CMP1309, CMP1331, CMP1330, CMP1333, CMP1329, CMP1184, and CMP1332.

[0454] PKL expressing strains were constructed by transforming strain CMP1133 (BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA) and selecting for colonies on Luria-Bertani plates containing 20 μg/ml kanamycin. The kanamycin marker was removed using the protocol recommended by the manufacturer (Gene Bridges, Heidelberg, Germany) to form the indicated strains (Table 4).

Table 4: Description of *E. coli* strains

Strain Name	Genotype
CMP1183	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1090 (pTrcPKL *Bifidobacterium longum*)
CMP1328	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1321 (pTrcPKL *Enterococcus gallinarum*)
CMP1366	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1364 (pTrcPKL *Clostridium acetobutylicum*)
CMP1182	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1029 (pTrcPKL *Lactobacillus reuteri*)
CMP1308	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1305 (pTrcPKL *Nostoc punctiforme*)
CMP1309	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1306 (pTrcPKL *Rhodopseudomonas palustris*)
CMP1331	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1324 (pTrcPKL *Pantoea*)
CMP1330	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1323 (pTrcPKL *Mucilaginibacter paludis*)
CMP1333	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1326 (pTrcPKL *Thermobifida fusca*)
CMP1329	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1322 (pTrcPKL *Bifidobacterium breve*)
CMP1184	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1184 (pTrcPKL *Lactobacillus paraplantarum*)
CMP1332	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1325 (pTrcPKL *Rahnella aquatilus*)

Example 16: Comparison of expression, solubility and enzymatic activity of phosphoketolases isolated from various diverse bacteria.

[0455] Strains expressing pTrcPKL *B. longum* (strain CMP1183), pTrcPKL *E. gallinarum* (strain CMP1328), pTrcPKL *C. acetobutylicum* (strain CMP1328), pTrcPKL *L. reuteri* (strain CMP1182), pTrcPKL *N. punctiforme* (strain CMP1308), pTrcPKL *R. palustris* (CMP1309), pTrcPKL *Pantaoea* (CMP1331), pTrcPKL *M. paludis* (CMP1330), and pTrcPKL *T. fusca* (CMP1333) were grown in LB media, induced at OD_{600} ~ 0.5 with 200μM IPTG, and induced for 4 hours at a temperature of 30°C or 34°C. Cells were harvested by centrifuging 4 ml culture broth at 3000 rpm for 10 minutes. Cell pellets were re-suspended in 2ml of 50mM MES, 50mM NaCl pH6.0 with 0.1% DNAase and 0.5mM AEBSF. The cell suspension was lysed using a french pressure cell at 14,000 psi (American Instrument Company). The lysate was then centrifuged at 15,000 RPM for 10 minutes at 4°C in an Eppendorf 5804R centrifuge. The supernatant and pellet were separated. The pellets were resuspended in the lysis 50mM MES, 50mM NaCl pH6.0 buffer. Supernatent and pellet samples were analyzed by 4-12% SDS-PAGE gel electrophoresis. Solubility was assessed by comparison of soluble versus pellet (insoluble) phosphoketolase fractions.

[0456] Results showed that *B. longum* PKL, *E. gallinarum* PKL, *C. acetobutylicum* PKL, *L. reuteri* PKL, *N. punctiforme* PKL, *R. palustris* PKL, and *T. fusca* PKL had a solubility of greater than 70% at a temperature of 30°C. Solubility of *Pantaoea* PKL and *M. paludis* PKL increased to about 50% at a temperature of 34°C (Table 5).

Table 5. Results of biochemical analysis

Rank	**Strain Description**	**Expression**	**Solubility (30°C/34°C)**
CMP1183	*Bifidobacterium longum*	Strong	>90%/~75%
CMP1328	*Enterococcus gallinarum*	Strong	>95%
CMP1366	*Clostridium acetobutylicum*	Good	>80-90%
CMP1182	*Lactobacillus reuteri*	Strong	>90%/<20%
CMP1308	*Nostoc punctiforme*	Good	~80%/<20%
CMP1309	*Rhodopseudomonas palustris*	Good	~80%/<20%
CMP1331	*Pantoea*	Good	~20%/~50%

CMP1330	*Mucilaginibacter paludis*	Good	~20%/~50%
CMP1333	*Thermobifida fusca*	Good	~80%

Example 17: Kinetic analysis of phosphoketolase isolated from *Bifidobacterium longum* and from *Enterococcus gallinarum.*

[0457] Phosphoketolase (PKL) from *B. longum* was purified for use in subsequent kinetic experiments. PKL from *B. longum* was expressed in a BL21 strain (CMP1183) of a pTrc His2B plasmid. The cells were grown in Luria-Bertani medium with 50 μg/ml carbenecillin at 34°C prior to induction. Following induction with 200 μM IPTG, cultures were transferred to a room temperature shaker for 5 hours. Cells were harvested by centrifugation at 10,000 rpm for 10 min, 4C. Cell pellets were stored at -80C prior to purification. For purification, *B. longum* PKL cell pellet were resuspended in 20 mM HEPES pH 7.0, 60 mM NaCl, 0.5 mM AEBSF, 0.5 mM $MgCl_2$, 0.1 mg/ml DNaseI. Cells were lysed by repeated passage through the french pressure cell and clarified by ultracentrifugation at 50,000 rpm for 30 minutes. Clarified lysate containing PKL from *B. longum* was initially loaded onto a MonoQ 10/100GL column (GE Healthcare) equilibrated in 50 mM Tris, 50 mM NaCl, pH 7 and eluted with a gradient to 50 mM Tris, 1 M NaCl, pH 7. Resulting fractions were analyzed by SDS-PAGE. *B. longum* PKL was further purified using a Superdex 200 10/300GL equilibrated in 50 mM Tris, 50 mM NaCL, pH 7 and MonoQ 10/300 GL at pH 6.0 using a buffer gradient from 50 mM MES, 50 mM NaCl to 1M NaCl. *B. longum* PKL was quantitated using A280 and a molar extinction coefficient determined of 149550 (determined by VectorNTI) and also by gel densitometry method. Purification using ion exchange and gel filtration chromatography produced > 95% apparent homogeneity of the PKL. Fractions containing *B. longum* PKL were pooled for use in assaying PKL activity by using the ferric hydroxamate assay.

[0458] Phosphoketolase (PKL) from *E. gallinarum* was purified for use in subsequent kinetic experiments. PKL from *E. gallinarum* was expressed in a BL21 strain (CMP1328) of a pTrc His2B plasmid. Cells were grown in LB medium with 50 ug/ml carbenecillin at 37C prior to induction. Following induction with 200 uM IPTG, cultures were transferred to a 30C shaker for 5 hours. Cells were harvested by centrifugation at 10,000 rpm for 10 min, 4C. Cell pellets were stored at -80C prior to purification. For purification, *E. gallinarum* PKL cell pellets were

resuspended in 50 mM MES pH 6.0, 50 mM NaCL, 0.5 mM AEBSF, 0.1 mg/ml DNaseI. Cells were lysed by repeated passage through a French press and clarified by ultracentrifugation at 50,000rpm for 60 min. Clarified lysate containing PKL from *E. gallinarum* was loaded onto a DEAE HiTrap FF column equilibrated in 50 mM MES, 50 mM NaCl, pH 6 and eluted with a gradient to 50 mM MES, 1M NaCl, pH 6. The resulting fractions were analyzed by SDS-PAGE. Fractions containing PKL were pooled and desalted using a G25 desalting column into 50 mM MES, 50 mM NaCL pH 6.0. Further purification was achieved using a MonoQ 10/100 GL column equilibrated in 50 mM MES, 50 mM NaCL, pH 6 with a salt gradient to 1M NaCl. Fractions containing PKL were pooled and analyzed by SDS PAGE, quantitation was achieved using A280 a molar extinction coefficient of 136980 determined by Vector NTI. Purification using ion exchange and gel filtration chromatography produced >95% apparent homogeneity of the PKL. Fractions containing *E. gallinarum* PKL were pooled for use in assaying PKL activity by using the ferric hydroxamate assay.

[0459] Pooled fractions containing either *B. longum PKL* or *E. gallinarum* PKL were assayed for PKL activity using ferric hydroxamate assay. The catalytic activities of the PKLs were measured using a scaled down version of hydroxamate assay described in L. Meile *et. al.*, *Bacteriol.*, 2001, 183:2929-2936 and Frey *et. al.*, *Bioorganic Chem.*, 2008, 36:121-127, which are incorporated herein in there entirety by reference. The assays were performed in a 96-well plate (Costar catalog #9017) format, at 37°C. Each 300 µl reaction contained 1mM TPP, 10 mM potassium phosphate pH 6.0, 50 mM MES pH 6, 10 mM MgCl2, 5 mM F6P and PKL at concentration of 250 nM. Time points were taken at various intervals. In order to stop the reaction 60 µl of the reaction mixture was mixed with 60 µl of 2M hydroxylamine at pH 6.5, incubated for 10 min at room temperature. Addition of 40 µl of 15% TCA, 40 µl of 4M HCl, and 40 µl of 5% $FeCl_3$ in 0.1 M HCl was used to precipitate the protein and allow AcP detection. The samples were then centrifuged at 3000 rpm for 10 min. A 200 µl sample of supernatant was transferred to a microtiter plate and a plate reader was used to measure A505. An AcP standard curve ranging between 12.5 and 0.2 mM was generated for quantitation. The Michaelis constant, K_M for PKL from *B. longum and E. gallinarum* were determined at a saturating concentration of Pi (20 mM) and with F6P concentrations ranging from 0.3 mM to 20 mM. The reaction was initiated with the addition of 250 nM (7 µg) of purified *B. longum* or 500 nM (4 µg) of *E. gallinarum* PKL. Absorbance changes associated with the amount of AcP formed were monitored at 505 nm and plotted against time to determine the rate of the PKL reactions.

[0460] Kinetic parameters were evaluated for *B. longum* and *E. gallinarum* PKL with respect to F6P at saturating concentrations of Pi, and for *E. gallinarum* PKL with respect to X5P at saturating concentrations of Pi. The reaction rates were fit to the Michaelis-Menten equation in order to calculate the kinetic constants (Table 6, Figure 43 and Figure 44). *B. longum* PKL had a greater K_M for F6P substrate of 21.16 mM, where as *E. gallinarum* PKL had a K_M of 2.86 mM for F6P substrate and of 5.81 mM for X5P substrate. The k_{cat} of *B. longum* PKL was 16.6 s^{-1}, and k_{cat} of *E. gallinarum* PKL was 1.4 s-1 with respect to F6P and 4.4 s^{-1} with respect to X5P.

Table 6. Summary of kinetic parameters

Enzyme	Temp	Enzyme (μM)	Enzyme (mg)	Substrate	Time measured	Km (mM)	Vmax (μM/sec)	kcat (sec-1)
B. longum PKL	37°C	0.25	6.94E-03	F6P	60	21.16	4.41	16.6
E. gallinarum PKL	37°C	0.5	4.01E-03	F6P	30	2.86	0.71	1.4
E. gallinarum PKL	37°C	0.5	4.01E-03	X5P	30	5.81	2.18	4.4

Example 18: Phosphoketolase isolated from *Bifidobacterium longum* has sedoheptulose-7-phosphate catalytic activity.

[0461] Cells expressing fructose 6-phosphate (positive control), ribose 5-phosphate (negative control), or sedoheptulose 7-phosphate alone or with *B. longum* phosphoketolase were grown and assayed for metabolite production by LC-MS detection.

[0462] Analysis of metabolite detection by LC-MS indicated that in cells only expressing fructose 6-phosphate (F6P), ribose 5-phosphate (R5P), or sedoheptulose 7-phosphate (S7P), metabolites F6P, R5P, or S7P, respectively, were primarily detected (Figure 45). Cells co-expressing R5P with *B. longum* phosphoketolase showed that it was primarily retained as R5P with some AcP production. In contrast, cells co-expressing F6P with *B. longum* phosphoketolase showed that F6P detection disappeared and the formation of AcP was detected (Figure 45). Similarly, cells co-expressing S7P with *B. longum* phosphoketolase showed that S7P detection disappeared and the formation of AcP was detected (Figure 45).

Example 19: Production of Mevalonate (MVA) in recombinant host cells expressing Phosphoketolase at small scale

[0463] Melavonate (MVA) producing *E. coli* strains were constructed by expressing phosphoketolase from *Bifidobacterium longum*, *Enterococcus gallinarum*, *Clostridium acetubutylicum, Nostoc, Rhodopseudomonas palustris, Pantoea,* or *Thermobifida fusca* in addition to genes that encode thiolase, HMG-CoA synthase and HMG-CoA reductase (Table 7). An MVA –producing strain that did not express a phophoketolase was used as control (Table 7). The phosphoketolase expressing strains were screened for phosphoketolase expression and mevalonic acid yield when grown in glucose as compared to a control strain not expressing phosphoketolase in a small scale experiement.

Table 7. MVA-producing strains expressing phophoketolase

Strain name	Genotype
CHL875	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pTrcHis2B (empty plasmid), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1319	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1090 (pTrc *B. longum* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1341	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1321 (pTrc *E. gallinarum* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1344	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1305 (pTrc *N. punctiforme* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1347	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1306 (pTrc *R.palustris* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1350	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1324 (pTrc *Pantoea* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1353	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1326 (pTrc *Thermobifida fusca* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)
EWL1359	BL21, GI1.2-gltA, yhfS-PyddV-ispA, pgl-, attB-, pCMP1364 (pTrc *C. acetobutylicum* PKL), pCHL416 (constitutive pCL Upper E. gallinarum Upper MVA)

(i) *Materials*

Modified TM3 Media Recipe without yeast extract and $MgSO_4$ (per liter fermentation medium):

[0464] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotic are added after pH adjustment and sterilization.

1000X Modified Trace Metal Solution (per liter):

[0465] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

Growth rate measurement

[0466] Shake tubes containing 3 ml LB media, with appropriate antibiotics, were inoculated with glycerol culture stocks. Cultures were incubated for approximately 15 hours at 30°C, 220 rpm. Supplemented TM3 media was prepared by combining TM3 media (without $MgSO_4$ and yeast extract), 1% Glucose, 8mM $MgSO_4$, 0.02% yeast extract and appropriate antibiotics. 2 mL of supplemented TM3 were inoculated in each well of a 48-well sterile block to a final OD_{600} of 0.2. Blocks were sealed with Breathe Easier membranes and incubated for 2 hours at 34°C, 600rpm. After 2 hours of growth, the OD_{600} was measured in the micro-titer plate and cells were induced with various concentrations of IPTG. OD_{600} reading was taken every hour after the IPTG induction for 4 hrs. OD_{600} was measurements were performed using a SpectraMax Plus190 (Molecular Devices). Cells were grown overnight and the OD_{600} was measured.

Glucose Measurement

[0467] Glucose samples were collected by centrifuging 300 µl of cell culture in the 96-well conical bottom plate and centrifuged for 10 min at 4°C, 3000rpm. The supernatant was diluted 10-fold in DI water and the glucose concentration was measured using a Glucose oxidase assay kit purchased from Pointe Scientific.

Mevalonate Measurement

[0468] Mevalonate samples were processed by combining and incubating 34 µl of 10% sulfuric acid and 300 µl of cell culture on ice for 10 min. After 10 minutes at 4°C the mixture was centrifuged for 10 min at 4°C, 3000rpm. 250 µl of supernatant was collected in the 96-well conical bottom plate and sealed with Zone-Free™ Films plate sealer for mevalonate measurement in HPLC. Mevalonate yield was determined by calculating amount of total mevalonate made for the amount for glucose utilized.

Protein Expression Analysis

[0469] A 50 µl sample of 4hrs post induction whole broth cell culture was boiled for 5 minutes at 95°C with 50 µl of 2X SDS sample buffer and 10 µl of sample was loaded in the 4–12% Bis-Tris gels for expression analysis. Purified phosphoketolase enzyme and pre-stained standard were added in each gel. Gels were stained with SimplyBlue Coomassie® G-250 stain and destained with deionized water.

Phosphoketolase Expression and Solubility Analysis

[0470] Cells were harvested by centrifuging 4 ml culture broth at 3000 rpm for 10 minutes. Cell pellets were re-suspended in 2ml of 100mM Tris, 100mM NaCl pH7.6 with 0.1% DNAase and 0.5mM AEBSF. The cell suspension was lysed using a french pressure cell at 14,000 psi (American Instrument Company). The lysate was then centrifuged at 15,000 RPM for 10 minutes at 4°C in an Eppendorf 5804R centrifuge. The supernatant and pellet were separated. The pellets were resuspended in the lysis buffer. Gel samples were prepared for both pellet and supernatant to perform electrophoresis. Using the iBlot® Dry Blotting System, proteins were transferred into a nitrocellulose membrane followed by immune-detection with rabbit *Bifido longum* phosphoketoalse anti-serum as a primary antibody at 1:10,000 dilution and

Alexa Fluor 488 goat anti-rabbit IgG as a detection antibody at 2μg/ml concentration according to the manufacturers protocol. Protein bands were detected using a Storm 860 Molecular Imager (Molecular Dynamics) using the blue fluorescence scanner screen and protein concentration was calculated using ImageQuant software.

(iii) *Results*

[0471] Analysis of MVA produced from engineered *E. coli* strains expressing *B. longum* PKL (EWL1319), *E. gallinarum* PKL (EWL1341), *Nostoc* PKL (EWL1344), *R. palustris* PKL (EWL1347), *Pantoea* PKL (EWL1350), or *T. fusca* PKL (EWL1353) demonstrated that increasing mevalonate yield correlated with increasing IPTG induction (Figure 46). Analysis of protein expression in whole cell lysates prepared from engineered strains expressing PKL showed that protein expression was induced by IPTG (Figure 47A-D). These findings show that increased melvanolate yield was a result of increasing phosphoketolase protein expression. Increased mevalonate yield was also observed in strains expressing *B. longum* PKL (EWL1319), *E. gallinarum* PKL (EWL1341), or *C. acetobutylicum* PKL (EWL1359) as compared to the MVA producing control strain not expressing PKL (CHL875). The increased mevalonate yield in strains expressing *B. longum* PKL (Figure 48A), *gallinarum* PKL (Figure 49A), or *C. acetobutylicum* PKL (Figure 50A) correlated with increased IPTG induction. The maximum mevalonate yield of *B. longum* PKL, *E. gallinarum* PKL, and *C. acetobutylicum* strains expressing phosphoketolase demonstrated an increased yield as compared to maximum mevalonate yield produced by the control strain CHL875. Analysis of protein expression in cell lysates prepared from engineered strains expressing PKL confirmed that *B. longum* PKL (Figure 48B and C), *gallinarum* PKL (Figure 49B), or *C. acetobutylicum* PKL (Figure 50B) expression was induced by IPTG. Further analysis of the supenatent and pellet fraction isolated from strains expressing *B. longum* PKL showed that the phosphoketolase was primarily in the soluble fraction (Figure 48B) as compared to the insoluble fraction (Figure 48C). These results are consistent with the conclusion that increased mevalonate yield was a result of increasing phosphoketolase expression.

Example 20: Production of Mevalonate (MVA) in recombinant host cells expressing phosphoketolase at 15-L Scale

[0472] Mevalonate (MVA) producing strains expressing phosphoketolase from *Enterococcus gallinarum* (strain EWL1341) and *Clostridium acetubutylicum* (strain EWL1359) were compared to an MVA producing strain not expressing phosphoketolase (strain CHL875) in a 15 Liter scale experiment. Cumulative MVA yield on glucose, instantaneous yield on glucose, volumetric productivity of MVA, specific MVA productivity and cell performance index (CPI) were measured and analyzed.

(i) *Materials*

Medium Recipe (per liter fermentation medium):

[0473] K2HPO4 7.5 g, MgSO4 * 7H2O 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, yeast extract 0.5 g, 50% sulphuric acid 1.6 mL, 1000X Modified Trace Metal Solution 1 ml. All of the components were added together and dissolved in Di H2O. This solution was heat sterilized (123°C for 20 minutes). The pH was adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Glucose 10 g, Vitamin Solution 8 mL, and antibiotics were added after sterilization and pH adjustment.

1000X Modified Trace Metal Solution (per liter):

[0474] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

Vitamin Solution (per liter):

[0475] Thiamine hydrochloride 1.0 g, D-(+)-biotin 1.0 g, nicotinic acid 1.0 g, pyridoxine hydrochloride 4.0 g. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with 0.22 micron filter.

Macro Salt Solution (per liter):

[0476] MgSO4 * 7H2O 296 g, citric acid monohydrate 296 g, ferric ammonium citrate 49.6 g. All components were dissolved in water, q.s. to volume and filter sterilized with 0.22 micron filter.

Feed solution (per kilogram):

[0477] Glucose 0.590 kg, Di H2O 0.393 kg, K2HPO4 7.4 g, and 100% Foamblast882 8.9 g. All components were mixed together and autoclaved. After autoclaving the feed solution, nutrient supplements are added to the feed bottle in a sterile hood. Post sterilization additions to the feed are (per kilogram of feed solution), Macro Salt Solution 5.54ml, Vitamin Solution 6.55ml, 1000X Modified Trace Metal Solution 0.82ml.

(ii) *Experimental procedure*

[0478] Mevalonate (MVA) production from a modified *E. coli (BL21)* host (CMP1133) expressing introduced genes from the mevalonate pathway and PKL isolated from either *E. gllinarum* (strain EWL1341) or *C. acetobutylicum* (strain EWL1359) was evaluated by growing the strains in fed-batch culture at the 15-L scale (Table 9). MVA production was compared to a modified *E. coli (BL21)* host (CMP1133) that expressed introduced genes from the mevalonate pathway but did not express a PKL (strain CHL875) to determine if any yield improvement can be attributed to the use of a phosphoketolase.

Table 9. List of strains assayed for MVA production

Strain Name	**Host**	**MVA plasmid**	**PKL plasmid**
CHL875 (Control)	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA (MD12-778)	pCHL416 (pCL-PL(1.6) Upper E.gallinarum)	pTrcHis2B (empty vector)
EWL1341	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA (MD12-778)	pCHL416 (pCL-PL(1.6) Upper E.gallinarum)	pCMP1321 (pTrc PKL_gallinarum)
EWL1359	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA(MD12-778)	pCHL416 (pCL-PL(1.6) Upper E.gallinarum)	pCMP1364 (pTrc PKL_acetobutylicum)

[0479] Mevalonate (MVA) formation from glucose at the desired fermentation pH (7.0) and temperature (34°C) was determined. To start each experiment, the appropriate frozen vial of the *E. coli* (BL21) strain was thawed and inoculated into a flask with tryptone-yeast

extract (LB) medium and the appropriate antibiotics. After the inoculum grew to an optical density of approximately 1.0, measured at 550 nm (OD_{550}), 500 mL was used to inoculate a 15-L bioreactor and bring the initial tank volume to 5 L.

[0480] Mevalonate producing strains were run in several production process conditions (Table 10). The batched media had glucose batched in at 9.7 g/L. Induction was achieved by adding isopropyl-beta-D-1-thiogalactopyranoside (IPTG). A shot of IPTG was added to the tank to bring the concentration to a specified level when the cells were at an OD_{550} of 6. Once the glucose was consumed by the culture, as signaled by a rise in pH, the glucose feed solution was fed to meet metabolic demands at rates less than or equal to 10 g/min. The fermentation was run long enough to determine the maximum cumulative mevalonate mass yield on glucose, a total of 60 to 64 hrs elapsed fermentation time.

[0481] The performance metrics of a control strain, (CHL875) were compared to experimental strains, EWL1341, EWL1359. The relevant performance metrics were cumulative MVA yield on glucose, instantaneous MVA yield on glucose, volumetric productivity of MVA, specific MVA productivity and cell performance index. The experimental strains (with phosphoketolase) were run in the same conditions as the control (no phosphketolase expression) to determine if any yield improvement can be attributed to the use of the phosphoketolase enzyme.

[0482] Overall yield was calculated using the following formula:

%wt Yield on glucose = MVA total (t)/[(Feed Wt(0)-Feed Wt(t)+83.5)*0.59)],

where 0.59 is the wt% of glucose in the glucose feed solution and 83.5 is the grams of this feed batched into the fermentor at t=0. Each feed had its weight % measured independently.

[0483] CPI was calculated using the following formula:

CPI = total grams MVA/ total grams dry cell weight

[0484] Oxygen, Nitrogen, and Carbon Dioxide levels in the offgas were determined independently by two mass spectrometers, an iSCAN (Hamilton Sundstrand), and a Hiden HPR20 (Hiden Analytical) mass spectrometer. Dissolved Oxygen in the fermentation broth is measured by sanitary, sterilizable probe with an optical sensor provided Hamilton Company.

The citrate, glucose, acetate, and mevalonate concentrations in the fermentor broth was determined in broth samples taken at 4 hour intervals by an HPLC analysis. Concentration in broth samples were determined by comparison of the refractive index response versus a previously generated calibration curve using standard of a known concentration.

HPLC INFORMATION

System: Waters Alliance 2695
Column: BioRad - Aminex HPX-87H Ion Exclusion Column 300mm x 7.8mm Catalog # 125-0140
Column Temperature: 50C
Guard column: BioRad - Microguard Cation H refill 30mm x 4.6mm Catalog # 125-0129
Running buffer: 0.01N H_2SO_4
Running buffer flow rate: 0.6 ml / min
Approximate running pressure: ~1100-1200 psi
Injection volume: 20 microliters
Detector: Refractive Index (Knauer K-2301)
Runtime: 26 minutes

Table 10. Production process conditions

Run Number	Strain Used	Target IPTG concentration upon addition (if any) (uM)
20120821	CHL875	0
20120976	CHL875	0
20120977	EWL1341	0
20120978	EWL1341	50
20120979	EWL1341	100
20121056	EWL1359	0
20121057	EWL1359	100
20121058	EWL1359	400
20121059	CHL875	100 (not expected to have an effect due to empty PKL vector)

(iii) *Results*

[0485] The strain expressing the *C.acetobutylicum* phosphoketolase (EWL1359, runs 20121056, 201201057, 20121058) achieved a higher cumulative % yield of MVA on glucose than the strain expressing no phosphoketolase (CHL875, run 20120821, 20120976, 20121059) (Table 11 and Figure 51). The MVA yield increase was noted when at least 100μM IPTG was added to the tank (20121057 and 20121058).

[0486] The strain expressing the *E.gallinarum* phosphoketolase (EWL1341, runs 20120977, 201200978, 20120979) achieved a higher cumulative % yield of MVA on glucose than the strain expressing no phosphoketolase (CHL875, runs 20120821, 20120976, 20121059) (Table 11 and Figure 52). The MVA yield increase was noted when at least 50μM IPTG was added to the tank (20120978 and 20120979).

Table 11. MVA Productivity Metrics

Strain Name / Run Number / μM IPTG	Max Cumulative % Yield of MVA on glucose (g/g %)	Overall MVA Volumetric Productivity at time of max overall MVA yield (g/L/hr)	Max Optical Density	CPI (Total g MVA / total gDCW) at time of max overall MVA yield	Peak Specific Productivity (mg MVA/L/hr/ OD)	Peak MVA Titer (gram MVA / Liter tank broth)
CHL875 / 20120821 / 0	28.9	1.92	115.6	3.7	54.4	109.0
CHL875 / 20120976 / 0	29.8	1.84	106.1	3.7	58.0	107.9
EWL1341 / 20120977 / 0	30.1	1.84	105.5	4.1	64.8	109.7
EWL1341 / 20120978 / 50	33.7	1.86	100.3	4.5	67.7	114.3
EWL1341 / 20120979 / 100	34.6	1.75	95.3	4.4	74.6	107.0
EWL1359 / 20121056 / 0	31.8	1.81	101.0	3.6	59.6	105.0
EWL1359 /	32.8	1.83	99.4	3.9	68.2	109.2

20121057 / 100						
EWL1359 / 20121058 / 400	34.6	1.39	71.1	4.3	64.5	89.9
CHL875 / 20121059 / 100	31.3	1.91	102.4	3.9	63.2	112.4

[0487] The MVA yield in all cases correlated with the amount of IPTG added (Figure 53). For a direct comparison, in runs where 100uM IPTG was added, both strains expressing phosphoketolase (20120979 and 20121057) achieved a significantly higher cumulative % yield of MVA on glucose than the strain expressing no phosphoketolase (CHL875, run 201201059) (Table 12 and Figure 53). The cumulative MVA yield (in both cases where phosphoketolase was expressed) correlated well with the phosphoketolase activity measured in the respective runs (Table 12 and Figure 54).

Table 12. MVA yield / Phosphoketolase Activity

Strain Name	Run Number	µM IPTG	Max Cumulative % Yield of MVA on glucose (g/g %)	Phosphoketolase activity (mmol AcetylP /L/hr/OD)
CHL875	20120821	0	28.9	Not measured
CHL875	20120976	0	29.8	Not measured
EWL1341	20120977	0	30.1	0.751
EWL1341	20120978	50	33.7	1.114
EWL1341	20120979	100	34.6	1.511
EWL1359	20121056	0	31.8	0.123
EWL1359	20121057	100	32.8	0.481
EWL1359	20121058	400	34.6	0.685
CHL875	20121059	100	31.3	Not measured

[0488] The strain expressing the *C.acetobutylicum* phosphoketolase (EWL1359, run 20121057, induced at 100µM IPTG) achieved a slightly higher cell performance index (g MVA / g dry cell weight) than the strain expressing no phosphoketolase (CHL875, run 20121059, induced at 100µM IPTG). When induced with 400µM IPTG (EWL1359, run 20121058), the CPI was higher than the control (CHL875, run 20121059, induced at 100µM IPTG) (Figure 55).

[0489] The strain expressing the *E.gallinarum* phosphoketolase (EWL1341, run 20120978 and 20120979, induced at 50μM IPTG and 100μM, respectively) achieved a higher cell performance index (g MVA / g dry cell weight) than the strain expressing no phosphoketolase (CHL875, run 20120976, no IPTG given). When no IPTG was added (EWL1341, run 20120977), the CPI was only slightly higher than the control (CHL875, run 20120976, no IPTG given) (Figure 56).

Example 21: Production of isoprene in recombinant host cells expressing phosphoketolase at small scale

[0490] Isoprene producing *E. coli* strains were constructed that expressed phosphoketolase from *Bifidobacterium longum*, *Enterococcus gallinarum* or *Clostridium acetobutylicum*. Isoprene producing strains that did not express a phophoketolase were used as controls (Table 13). The phosphoketolase expressing strains were screened for phosphoketolase expression and isoprene yield when grown in glucose as compared to a control strain not expressing phosphoketolase in a small scale experiment.

[0491] Isoprene producing strains were made in a modified *E. coli (BL21)* host (MCM2065) expressing *B. longum* PKL were made by introducing plasmid pEWL1418 (Figure 57) to produce strain EWL1427 (Table 13). For production of strains expressing *E. gallinarum* PKL, plasmid pEWL1421 (Figure 58) or pEWL1438 (Figure 59) was introduced into a modified *E. coli (BL21)* host (MCM2065) to generate strain EWL1430 and strain EWL1449, respectively (Table 13). For production of strains expressing *C. acetobutylicum* PKL, plasmid pEWL1436 (Figure 60) and plasmid pEWL1440 (Figure 61) were used to produce strain EWL1446 and strain EWL1452, respectively (Table 13).

Table 13. Isoprene-producing strains expressing phophoketolase

Strain name	Genotype
MCM2158 (Control)	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK + pTrc P.alba(MEA variant)-MVKdel + pMCM1225 (inducible pCL E. gal Upper MVA)
EWL1427	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK + pTrc P.alba(MEA variant)-*B. longum* PKL + pMCM1225 (inducible pCL E. gal Upper MVA)

EWL1430	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK + pTrc P.alba(MEA variant)-*E. gallinarum* PKL + pMCM1225 (inducible pCL E. gal Upper MVA)
EWL1446	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK, + pTrc P.alba(MEA variant)-*C.acetobutylicum* PKL + pMCM1225 (inducible pCL E. gal Upper MVA)
DW719 (Control)	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pTrc P.alba(MEA variant)-mMVK + pMCM1225 (inducible pCL E. gal Upper MVA)
EWL1449	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pTrc P.alba(MEA variant)-mMVK-*E.gallinarum* PKL + pMCM1225 (inducible pCL E. gal Upper MVA)
EWL1452	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pTrc P.alba(MEA variant)-mMVK-*C. acetobutylicum* PKL + pMCM1225 (inducible pCL E. gal Upper MVA)

bMVK indicates *M. burtonii* mevalonate kinase
mMVK indicates *M. mazei* mevalonate kinase

(i) *Materials*

TM3 Media Recipe (per liter fermentation medium):

[0492] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotic are added after pH adjustment and sterilization.

Modified TM3 Media Recipe without yeast extract and $MgSO_4$ (per liter fermentation medium):

[0493] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotic are added after pH adjustment and sterilization.

1000X Modified Trace Metal Solution (per liter):

[0494] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

Growth rate measurement

[0495] Shake tubes containing 3 ml LB media, with appropriate antibiotics, were inoculated with glycerol culture stocks. Cultures were incubated for approximately 15 hours at 30°C, 220 rpm. Supplemented TM3 media was prepared by combining TM3 media (without $MgSO_4$ and yeast extract), 1% Glucose, 8mM $MgSO_4$, 0.02% yeast extract and appropriate antibiotics. 2 mL of supplemented TM3 were inoculated in each well of a 48-well sterile block to a final OD_{600} of 0.2. Blocks were sealed with Breathe Easier membranes and incubated for 2 hours at 34°C, 600rpm. After 2 hours of growth, the OD_{600} was measured in the micro-titer plate and cells were induced with various concentrations of IPTG. OD_{600} reading was taken every hour after the IPTG induction for 4 hrs. OD_{600} was measurements were performed using a SpectraMax Plus190 (Molecular Devices). Cells were grown overnight and the OD_{600} was measured.

Glucose Measurement

[0496] Glucose samples were collected by centrifuging 300 µl of cell culture in the 96-well conical bottom plate and centrifuged for 10 min at 4°C, 3000rpm. The supernatant was diluted 10-fold in DI water and the glucose concentration was measured using a Glucose oxidase assay kit purchased from Pointe Scientific.

Isoprene Specific Productivity Measurement

[0497] A 100 µl of isoprene sample was collected in a 96-well glass block every hour after IPTG induction for 4 hours. The glass block was sealed with aluminum foil and incubated at 34°C while shaking at 450 rpm, for 30 minutes using a Thermomixer. After 30 minutes, the

block was kept at 70°C water bath for 2 minutes and levels of isoprene in the headspace measurement were determined using gas chromatography-mass spectrometry.

Protein Expression Analysis

[0498] A 50 µl sample of 4hrs post induction whole broth cell culture was boiled for 5 minutes at 95°C with 50 µl of 2X SDS sample buffer and 10 µl of sample was loaded in the 4–12% Bis-Tris gels for expression analysis. Purified phosphoketolase enzyme and pre-stained standard were added in each gel. Gels were stained with SimplyBlue Coomassie® G-250 stain and destained with deionized water.

(iii) *Results*

[0499] Analysis of isoprene produced from glucose by engineered *E. coli* strains expressing *B. longum* PKL in the presence of *M. burtonii* mevalonate kinase expression (strain EWL1427), *E. gallinarum* PKL in the presence of *M. burtonii* mevalonate kinase expression (strain EWL1430), or *C. acetobutylicum* PKL in the presence of *M. burtonii* mevalonate kinase expression (strain EWL1446) demonstrated that increasing isoprene yield correlated with increasing IPTG induction as compared to a control strain that did not express a PKL (strain MCM2158) (Figure 62A and B). Analysis of protein expression in whole cell lysates prepared from engineered strains expressing PKL showed that protein expression was induced by IPTG (Figure 63A and B). These findings indicate that increased isoprene yield was a result of increasing phosphoketolase protein expression.

[0500] Analysis of isoprene produced from glucose by engineered *E. coli* strains expressing *E. gallinarum* PKL in the presence of *M. mazei* mevalonate kinase expression (strain EWL1449) or *C. acetobutylicum* PKL in the presence of *M. mazei* mevalonate kinase expression (strain EWL1452) demonstrated that increasing isoprene yield correlated with increasing IPTG induction as compared to a control strain that did not express a PKL (strain DW719) (Figure 64A). Analysis of protein expression in whole cell lysates prepared from engineered strains expressing PKL showed that protein expression was induced by IPTG (Figure 64B). These findings indicate that increased isoprene yield was a result of increasing phosphoketolase protein expression.

[0501] Overall these results demonstrated the production of isoprene from glucose in strains that express PKL genes isolated from different bacteria such as *B. longum*, *E. gallinarum*, and *C. acetobutylicum* in the presence of different mevalonate kinase genes.

Example 22: Production of isoprene in recombinant host cells expressing phosphoketolase at 15-L scale

[0502] Isoprene producing strains expressing phosphoketolase from *B. longum* (strain EWL1427) or *E. gallinarum* (strain EWL1430) were compared to an isoprene producing strain not expressing phosphoketolase (strain MCM2158) in a 15 Liter scale experiment for production of isoprene. Cumulative isoprene yield on glucose, instantaneous isoprene yield on glucose and cell performance index (CPI) were measured and analyzed.

(i) *Materials*

Medium Recipe (per liter fermentation medium):

[0503] K2HPO4 7.5 g, MgSO4 * 7H2O 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, yeast extract 0.5 g, 50% sulphuric acid 1.6 mL, 1000X Modified Trace Metal Solution 1 ml. All of the components were added together and dissolved in Di H2O. This solution was heat sterilized (123°C for 20 minutes). The pH was adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Glucose 10 g, Vitamin Solution 8 mL, and antibiotics were added after sterilization and pH adjustment.

1000X Modified Trace Metal Solution (per liter):

[0504] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

Vitamin Solution (per liter):

[0505] Thiamine hydrochloride 1.0 g, D-(+)-biotin 1.0 g, nicotinic acid 1.0 g, pyridoxine hydrochloride 4.0 g. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with 0.22 micron filter.

Macro Salt Solution (per liter):

[0506] MgSO4 * 7H2O 296 g, citric acid monohydrate 296 g, ferric ammonium citrate 49.6 g. All components were dissolved in water, q.s. to volume and filter sterilized with 0.22 micron filter.

Feed solution (per kilogram):

[0507] Glucose 0.590 kg, Di H2O 0.393 kg, K2HPO4 7.4 g, and 100% Foamblast882 8.9 g. All components were mixed together and autoclaved. After autoclaving the feed solution, nutrient supplements are added to the feed bottle in a sterile hood. Post sterilization additions to the feed are (per kilogram of feed solution), Macro Salt Solution 5.54ml, Vitamin Solution 6.55ml, 1000X Modified Trace Metal Solution 0.82ml.

(ii) *Experimental procedure*

[0508] Isoprene production from a modified *E. coli (BL21)* host (MCM2065) expressing introduced genes from the mevalonate pathway and PKL isolated from either *B. longum* (strain EWL1427) or *E. gallinarum* (strain EWL1430) was evaluated by growing the strains in fed-batch culture at the 15-L scale (Table 14). Isoprene production was compared to a modified *E. coli (BL21)* host (MCM2065) that expressed introduced genes from the mevalonate pathway but did not express a PKL (strain MCM2158) to determine if any yield improvement could be attributed to the use of a phosphoketolase.

Table 14. List of isoprene producing strains

Strain Name	Host	Upper pathway MVA plasmid	Isoprene synthase / PKL plasmid	Run numbers
MCM2158 (Control)	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIsp AyhfS, thiFRTtruncIspA, bMVK (MCM2065)	inducible *E.gallinarum* Upper (pMCM1225 Spec 50)	pTrcP.alba(MEA variant) minusMVK/ no PKL gene on this plasmid	20121134
EWL1427	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIsp AyhfS, thiFRTtruncIspA,	pMCM1225 inducible *E.gallinarum* Upper (pMCM1225 Spec 50)	pTrc P.alba(MEA variant) minusMVK +B.longum PKL (pEWL1418 Carb50)	20121135

	bMVK (MCM2065)			
EWL1430	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIsp AyhfS, thiFRTtruncIspA, bMVK (MCM2065)	pMCM1225 inducible *E.gallinarum* Upper (pMCM1225 Spec 50)	pTrc P.alba(MEA variant) minusMVK +E.gal PKL (pEWL1421 Carb50)	20121136

bMVK indicates *M. burtonii* mevalonate kinase

[0509] Isoprene producing strains were run in a standard production process (Table 15). Isoprene production from glucose was monitored at the fermentation pH 7.0 and temperature of 34°C. To initiate the experiment, a frozen vial of the *E. coli* (BL21) strain was thawed and inoculated into a flask with tryptone-yeast extract (LB) medium and the appropriate antibiotics. After the inoculum grew to an optical density of approximately 1.0, measured at 550 nm (OD_{550}), 500 mL was used to inoculate a 15-L bioreactor and bring the initial tank volume to 5 L. The batched media had glucose batched in at 9.7 g/L. Induction was achieved by adding isopropyl-beta-D-1-thiogalactopyranoside (IPTG). A shot of IPTG was added to the tank to bring the concentration to a specified level when the cells were at an OD_{550} of 6. Once the glucose was consumed by the culture, as signaled by a rise in pH, the glucose feed solution was fed to meet metabolic demands at rates less than or equal to 10 g/min. The fermentation was run long enough to determine the maximum cumulative isoprene mass yield on glucose for a total of 60 to 64 hrs elapsed fermentation time.

Table 15. Production process conditions

Run Number	Strain Used	Target IPTG concentration upon tank addition (μM)	Target IPTG concentration in feed bottle (μM)
20121134	MCM2158	100	100
20121135	EWL1427	100	100
20121136	EWL1430	100	100

[0510] The performance metrics of a control strain, (MCM2158) were compared to experimental strains, EWL1427 and EWL1430. The performance metrics were cumulative isoprene yield on glucose, instantaneous isoprene yield on glucose and cell performance index

(CPI). The experimental strains (with phosphoketolase) were run in the same conditions as the control (no phosphketolase expression).

[0511] Overall isoprene yield was calculated using the following formula:

%wt Yield on glucose = Isoprene total (t)/[(Feed Wt(0)-Feed Wt(t)+83.5)*0.59)],

where 0.59 is the wt% of glucose in the glucose feed solution and 83.5 is the grams of this feed batched into the fermentor at t=0. Each feed had its weight % measured independently.

[0512] Isoprene Instantaneous yield was calculated using the following formula:

Isoprene Inst. yield (g/g%) = Isoprene produced (t_1-t_0)/consumed glucose (t_1-t_0)*100

[0513] CPI was calculated using the following formula:

CPI = total grams Isoprene/ total grams dry cell weight

[0514] Isoprene, Oxygen, Nitrogen, and Carbon Dioxide levels in the off-gas were determined independently by two mass spectrometers, an iSCAN (Hamilton Sundstrand), and a Hiden HPR20 (Hiden Analytical) mass spectrometer. Dissolved Oxygen in the fermentation broth is measured by sanitary, sterilizable probe with an optical sensor provided Hamilton Company. The citrate, glucose, acetate, and mevalonate concentrations in the fermentor broth was determined in broth samples taken at 4 hour intervals by an HPLC analysis. Concentration in broth samples were determined by comparison of the refractive index response versus a previously generated calibration curve using standard of a known concentration.

HPLC INFORMATION

System: Waters Alliance 2695
Column: BioRad - Aminex HPX-87H Ion Exclusion Column 300mm x 7.8mm Catalog # 125-0140
Column Temperature: 50C
Guard column: BioRad - Microguard Cation H refill 30mm x 4.6mm Catalog # 125-0129
Running buffer: 0.01N H_2SO_4
Running buffer flow rate: 0.6 ml / min
Approximate running pressure: ~1100-1200 psi
Injection volume: 20 microliters
Detector: Refractive Index (Knauer K-2301)
Runtime: 26 minutes

(iii) *Results*

[0515] The strain expressing the *E.gallinarum* phosphoketolase (EWL1430, 20121136) achieved a higher cumulative % yield of isoprene on glucose than the strain expressing no phosphoketolase (MCM2158, 20121134) (Table 16 and Figure 65). The strain expressing the *E.gallinarum* phosphoketolase (EWL1430, 20121136) achieved a higher instantaneous % yield of isoprene on glucose than the strain expressing no phosphoketolase (MCM2158, 20121134) (Table 16 and Figure 66) and maintained the high yield for a longer period of time resulting in a higher cumulative yield (Table 16 and Figure 65). Though the strain expressing the *B.longum* phosphketolase (EWL1427, 20121135) had a higher peak instantaneous yield late in the run (Figure 66), this strain took longer to attain the peak yield and in the end finished with approximately about the same cumulative yield of isoprene on glucose as the control strain (MCM2158, 20121134) (Table 16 and Figure 65)

[0516] The strain expressing the *E.gallinarum* phosphoketolase (EWL1430, 20121136) achieved a slightly higher CPI than the strain expressing no phosphoketolase (MCM2158, 20121134) (Figure 67). The strain expressing the *B.longum* phosphketolase (EWL1427, 20121135) had a CPI that was slightly lower than the strain expressing no phosphoketolase (MCM2158, 20121134) (Figure 67). The time of peak cumulative yield (60hrs EFT) was the time point where the CPI was compared.

Table 16. Isoprene Productivity Metrics

Strain Name / Run Number / [IPTG] in batch (µM)	Max Cumulative % Yield of isoprene on glucose (g/g %)	Overall Isoprene Volumetric Productivity at time of max overall isoprene yield (g/L/hr)	Max Optical Density	CPI (Total g isoprene / total gDCW) at time of max overall isoprene yield	Peak Specific Productivity (mg isoprene/L/ hr/OD)	Peak Instantaneous yield of isoprene on glucose (g/g%)	Peak isoprene Titer (gram isoprene / average volume of tank broth in Liters)
MCM2158 / 20121134 / 100	16.3	2.21	103.6	2.5	41.1	19.9	123.6
EWL1427 / 20121135 / 100	16.2	1.53	123.9	2.2	26.6	21.9	85.4
EWL1430 / 20121136 / 100	17.4	1.94	105.8	2.6	39.2	21.4	108.8

Table 17. Summary of Isoprene yield

Strain Name	Run Number	[IPTG] (μM)	Max Cumulative % Yield of isoprene on glucose (g/g %)
MCM2158	20121134	100	16.3
EWL1427	20121135	100	16.2
EWL1430	20121136	100	17.4

Table 18. Summary of PKL specific productivities in units of AcP formation and specific activity of PKL from *B. longum* and *E. gallinarum.*

Fermentation Run	Strain	IPTG induction (μM)	EFT (h)	Specific Productivity of AcP (mmol/L/h/OD)	PKL Specific Activity (μmol/mg/min)
F20121134	MCM2158	100	24	0.06	5.9E-03
F20121135	EWL1427	100	24	7.30	7.1E-01
			36	9.41	8.7E-01
			48	10.01	8.1E-01
F20121136	EWL1430	100	24	0.98	8.0E-02
			36	1.09	1.0E-01
			48	1.13	9.9E-02

Example 23: I*n vitro* specific activity analysis of phosphoketolase in recombinant host cells grown expressing cells grown at 14L Scale

[0517] Strains expressing phosphoketolase (PKL) from *E. gallinarum* (Table 19 and Table 21), *C. acetobutylicum* (Table 20), or *B. longum* (Table 21) were assayed in kinetic experiments. For preparation of the samples, cell pellets were obtained from one mL of culture during the course of 14L fermentation runs and stored at -80°C. Pellet samples were resuspended and normalized to OD(600)=20 in 50 mM MES, pH 6 with 1 mg/ml lysozyme, 0.2 mg/ml DNaseI and 0.5 mM AEBSF. OD normalized cell pellets were lysed by repeated passage through a French Pressure Mini Cell at 700 psi set to medium ratio. Lysed samples were then clarified by centrifugation at 14,000 rpm for 10 min at 4°C. Activity assays and protein measurements were preformed on the soluble lysate fraction. Total protein was determined by

Bradford Bio Rad method using a standard curve prepared by titrating BSA at concentrations ranging between 0.5 and 0.05 mg/ml.

[0518] The catalytic activities of the PKLs were measured using a scaled down version of hydroxamate assay described in L. Meile *et. al.*, *Bacteriol.*, 2001, 183:2929-2936 and Frey *et. al.*, *Bioorganic Chem.*, 2008, 36:121-127, which are incorporated in their entirety herein by reference. The assays were performed in a 96-well plate (Costar catalog #9017) format, at 37°C. In the standard assay, the reaction mixture consisted of 5 mM F6P, 1 mM TPP, 10 mM potassium phosphate, 50 mM MES buffer at pH 6, and 10 mM $MgCl_2$, 20 mM sodium fluoride, 8 mM iodoacetomide and 1 mM DTT. The reaction is started with the addition of F6P and stopped after 30 minutes of incubation. Total reaction volume is usually 300 ul (smaller amounts where used when necessary). AcP was used as a standard curve with concentrations ranging between 15-0.23 mM. In order to stop the reaction, 60 µl of the reaction mixture was mixed with 60 µl of 2M hydroxylamine at pH 6.5, incubated for 10 min at room temperature. Addition of 40 µl of 15% TCA, 40 µl of 4M HCl, and 40 µl of 5% $FeCl_3$ in 0.1 M HCl was used to precipitate the protein and allow AcP detection. The samples were then centrifuged at 3000 rpm for 10 min. 200 µl of the supernatant was transferred to a microtiter plate in order to measure the rates of AcP formation at absorbance of 505 nm.

Table 19. 14L scale runs with *E. gallinarum* PKL expressed in a constitutive mevalonate background

Fermentation run	**Strain**	**Description**	**IPTG induction (µM)**
20120977	EWL1341	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1321 (pTrc PKL_E. gallinarum)	0
20120978	EWL1341	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1321 (pTrc	50

		PKL_E. gallinarum)	
20120979	EWL1341	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1321 (pTrc PKL_E. gallinarum)	100

Table 20. 14L scale runs with *C. acetobutylicum* PKL expressed in a constitutive upper pathway strain

Fermentation run	**Strain**	**Description**	**IPTG induction (μM)**
20121056	EWL1359	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1364 (pTrc PKL_C. acetobutylicum)	0
20121057	EWL1359	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1364 (pTrc PKL_C. acetobutylicum)	100
20121058	EWL1359	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, attB-pCHL416 (constitutive PL.6-E. gallinarum upper), pCMP1364 (pTrc PKL_C. acetobutylicum)	400

Table 21. 14L scale runs with *E. gallinarum* PKL or B. longum PKL expressed in a strain with a full mevalonate pathway and isoprene synthase

Fermentation run	Strain	Description	IPTG induction (μM)
20121134	MCM2158	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, FRT-MVK(burtonii) + pTrcP.alba (MEA variant)-MVKdel2 + pCL-Ptrc-Upper_Egallinarum)	100
20121135	EWL1427	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK + pEWL1418 (pTrc P.alba IspS MEA variant)-B.longum PKL) + pMCM1225)	100
20121136	EWL1430	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, bMVK + pEWL1421 (pTrc P.alba IspS MEA variant)-E.gallinarum PKL) + pMCM1225	100

[0519] In vitro specific productivities of AcP formation for EWL1341 strain harboring *E. gallinarum* PKL ranged from 0.75 to 1.77 mmol/L/h/OD depending on the level of induction (Table 22 and Figure 68A). Furthermore, in vitro specific activities of AcP formation for EWL1341 strain harboring *E. gallinarum* PKL ranged from 7.1E-02 and 1.1E-01μmol/mg/min depending on the level of induction (Table 22 and Figure 68B).

Table 22. Summary of *E. gallinarum* PKL specific productivities in units of AcP formation and specific activity of *E. gallinarum* PKL.

Fermentation Run	Strain	IPTG induction (μM)	EFT (h)	Specific Productivity of AcP (mmol/L/h/OD)	PKL Specific Activity (μmol/mg/min)

F20120977	EWL1341	0	24	0.88	8.8E-02
			36	0.75	7.1E-02
F20120978	EWL1341	50	24	1.27	9.0E-02
			36	1.11	7.6E-02
F20120979	EWL1341	100	24	1.77	1.1E-01
			36	1.51	8.0E-02

[0520] In vitro specific productivities of AcP formation for EWL1359 strain harboring *C. acetobutylicum* PKL ranged from 0.12 to 0.74 mmol/L/h/OD depending on the level of induction (Table 23 and Figure 69A). Furthermore, in vitro specific activities of AcP formation for EWL1359 strain harboring *C. acetobutylicum* PKL ranged from 1.1E-02 and 7.6E-02 μmol/mg/min depending on the level of induction (Table 23 and Figure 69B).

Table 23. Summary of *C. acetobutylicum* PKL specific productivities in units of AcP formation and specific activity of *C. acetobutylicum* PKL.

Fermentation Run	Strain	IPTG induction (μM)	EFT (h)	Specific Productivity of AcP (mmol/L/h/OD)	PKL Specific Activity (μmol/mg/min)
F20121056	EWL1359	0	24	0.15	1.4E-02
			36	0.12	1.2E-02
			48	0.10	1.1E-02
F20121057	EWL1359	100	24	0.48	4.3E-02
			36	0.40	3.5E-02
			48	0.36	3.3E-02
F20121058	EWL1359	400	24	0.74	7.6E-02
			36	0.69	6.8E-02
			48	0.62	6.5E-02

[0521] In vitro specific productivities of AcP formation for EWL1427 strain harboring *B. longum* PKL and induced with 100 μM IPTG ranged from 7.30 to 10.01 mmol/L/h/OD (Table 23 and Figure 70). In vitro specific productivities of AcP formation for EWL1430 strain harboring *E. gallinarum* PKL ranged from 0.98 to 1.13 mmol/L/h/OD when induced with 100 μM IPTG and from 1.30 to 1.38 mmol/L/h/OD when induced with 200 μM IPTG (Table 24 and Figure 70).

[0522] In vitro specific activities of AcP formation for EWL1427 strain harboring *B. longum* PKL and induced with 100 μM IPTG ranged from 7.1E-01 to 8.7E-01 μmol/mg/min (Table 23 and Figure 71). In vitro specific productivities of AcP formation for EWL1430 strain harboring *E. gallinarum* PKL ranged from 8.0E-02 to 1.0E-01 μmol/mg/min when induced with 100 μM IPTG and from 1.1E-01 to 1.2E-01 μmol/mg/min when induced with 200 μM IPTG (Table 24 and Figure 71).

[0523] Overall, these findings indicate that phophoketolase activity was present in all strains.

Table 24. Summary of PKL specific productivities in units of AcP formation and specific activity of PKL from *B. longum* and *E. gallinarum*.

Fermentation Run	Strain	IPTG induction (μM)	EFT (h)	Specific Productivity of AcP (mmol/L/h/OD)	PKL Specific Activity (μmol/mg/min)
F20121134	MCM2158	100	24	0.06	5.9E-03
F20121135	EWL1427	100	24	7.30	7.1E-01
			36	9.41	8.7E-01
			48	10.01	8.1E-01
F20121136	EWL1430	100	24	0.98	8.0E-02
			36	1.09	1.0E-01
			48	1.13	9.9E-02

Example 24: Construction of phosphoketolase-expressing strains harboring host mutations for producing isoprene.

[0524] Isoprene-producing strains comprising an active phosphoketolase polypeptide as described above can be further engineered to increase the activity of one or more of the following genes including ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*), D-ribulose-5-phosphate 3-epimerase (*rpe*), transketolase (*tktA* and/or *tktB*), transaldolase B (*tal B*), phosphoenolpyruvate synthetase (*ppsA*), phosphate acetyltransferase (*pta* and/or *eutD*) to improve carbon flux through the phosphoketolase pathway (Figure 72). In certain aspects, the activity of the following genes rpiA, rpiB, rpe, tktA, tktB, tal B, ppsA, eutD, and/or pta can be increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In one embodiment the activity of ribose-5-phosphate isomerase (*rpiA* and/or *rpiB*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a

plasmid. In another embodiment the activity of D-ribulose-5-phosphate 3-epimerase (*rpe*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In another embodiment the activity of transketolase (*tktA* and/or *tktB*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In yet another embodiment the activity of transaldolase B (*tal B*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In another embodiment the activity of phosphoenolpyruvate synthetase (*ppsA*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In still other embodiments the activity of phosphate acetyltransferase (*pta* and/or *eutD*) is increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid. In certain aspects, isozymes of the following genes rpiA, rpiB, rpe, tktA, tktB, tal B, ppsA, eutD, and/or pta can be increased by altering the promoter and/or rbs on the chromosome, or by expressing it from a plasmid.

[0525] These strains can be further engineered to decrease the activity of one or more of the following genes including glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphofructokinase-1 (*pfkA* and/or *pfkB*), fructose bisphosphate aldolase (*fba*, *fbaA*, *fbaB*, and/or *fbaC*), glyceraldehyde-3-phosphate dehydrogenase (*gapA* and/or *gapB*), acetate kinase (*ackA*), citrate synthase (*gltA*), transketolase (*tktA* and/or *tktB*), EI (*ptsI*), $EIICB^{Glc}$ (*ptsG*), $EIIA^{Glc}$ (*crr*), and/or HPr (*ptsH*) to increase carbon flux into the phosphoketolase pathway (Figure 73). In one embodiment, a *zwf* gene encoding glucose-6-phosphate dehydrogenase is downregulated. In another embodiment, a *pfkA* gene encoding 6-phosphofructokinase-1 A is downregulated. In another embodiment, a *gapA* gene encoding glyceraldehyde-3-phosphate dehydrogenase A is downregulated. In another embodiment, a *fba* gene encoding fructose bisphosphate aldolase is downregulated. In yet another embodiment, a *gltA* gene encoding citrate synthase is downregulated. In an embodiment,a *ackA* gene encoding acetate kinase is downregulated. In another embodiment, a *ptsI* gene encoding EI is downregulated. In an embodiment, a *ptsH* gene encoding HPr is downregulated. In another embodiment, a *ptsG* gene encoding $EIICB^{Glc}$ is downregulated. In a yet another embodiment, a *crr* gene encoding $EIIA^{Glc}$ is downregulated. The pts operon encodes genes of the phosphotransferase system. In some embodiments, the strains can be engineered to decrease activity of the phosphotransferase system (PTS) to increase carbon flux into the phosphoketolase pathway. In some embodiments, the PTS is downregulated by downregulation of the pts operon. In certain aspects, the PTS is downregulated and a glucose transport pathway is upregulated. A glucose transport pathway

includes, but is not limited to, galactose (*galP*) and glucokinase (*glk*) genes. In some embodiments, the pts operon is downregulated, the galactose (*galP*) gene is upregulated, and the glucokinase (*glk*) gene is upregulated. In certain aspects, isozymes of proteins encoded by the following genes zwf, pfkA, fba, gapA, ackA, gltA, tktA, ptsG, ptsH, ptsI, and/or crr can be downregulated to increase carbon flux into the phosphoketolase pathway. In some embodiments, the pfkB gene is downregulated. In some embodiments, the glyceraldehyde-3-phosphate dehydrogenase B (gapB) gene is downregulated. In some embodiments, the transketolase B (tktB) gene is downregulated.

Example 25: Production of isoprene by phosphoketolase-expressing strains harboring host mutations at small scale.

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0526] K_2HPO_4 13.6 g, KH_2PO_4 13.6 g, $MgSO_4*7H_2O$ 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, $(NH_4)_2SO_4$ 3.2 g, yeast extract 0.2 g, 1000X Trace Metals Solution 1 ml. All of the components are added together and dissolved in diH_2O. The pH is adjusted to 6.8 with ammonium hydroxide (30%) and brought to volume. Media is filter-sterilized with a 0.22 micron filter. Glucose 10.0 g and antibiotics are added after pH adjustment and sterilization.

1000X Trace Metal Solution (per liter fermentation media)

[0527] Citric Acid*H_2O 40g, $MnSO_4*H_2O$ 30g, NaCl 10g, $FeSO_4*7H_2O$ 1g, $CoCl_2*6H_2O$ 1g, $ZnSO_4*7H_2O$ 1g, $CuSO_4*5H_2O$ 100mg, H_3BO_3 100mg, $NaMoO_4*2H_2O$ 100mg. Each component is dissolved one at a time in diH_2O. The pH is adjusted to 3.0 with HCl/NaOH, and then the solution is brought to volume and filter-sterilized with a 0.22 micron filter.

(ii) *Experimental procedure*

[0528] Cells are grown overnight in Luria-Bertani broth + antibiotics. The day after, they are diluted to an OD600 of 0.1 in 20 mL TM3 medium containing 50 ug/ml of spectinomycin, 25 ug/mL chloramphenicol and 50 ug/mL carbenicillin (in a 250-mL baffled

Erlenmeyer flask), and incubated at 34°C and 200 rpm. After 2h of growth, OD600 is measured and 200 uM IPTG is added. Samples are taken regularly during the course of the fermentation. At each timepoint, OD600 is measured. Also, off-gas analysis of isoprene is performed using a gas chromatograph-mass spectrometer (GC-MS) (Agilent) headspace assay. One hundred microliters of whole broth are placed in a sealed GC vial and incubated at 34°C and 200 rpm for a fixed time of 30 minutes. Following a heat kill step, consisting of incubation at 70°C for 7 minutes, the sample is loaded on the GC. The reported specific productivity is the amount of isoprene in ug/L read by the GC divided by the incubation time (30 min) and the measured OD600.

Example 26: Production of isoprene by phosphoketolase-expressing strains harboring host mutations at 15-L scale.

(i) *Materials*

Medium Recipe (per liter fermentation medium):

[0529] K2HPO4 7.5 g, MgSO4 * 7H2O 2 g, citric acid monohydrate 2 g, ferric ammonium citrate 0.3 g, yeast extract 0.5 g, 50% sulphuric acid 1.6 mL, 1000X Modified Trace Metal Solution 1 ml. All of the components were added together and dissolved in Di H2O. This solution was heat sterilized (123°C for 20 minutes). The pH was adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Glucose 10 g, Vitamin Solution 8 mL, and antibiotics were added after sterilization and pH adjustment.

1000X Modified Trace Metal Solution (per liter):

[0530] Citric Acids * H2O 40 g, MnSO4 * H2O 30 g, NaCl 10 g, FeSO4 * 7H2O 1 g, CoCl2 * 6H2O 1 g, ZnSO * 7H2O 1 g, CuSO4 * 5H2O 100 mg, H3BO3 100 mg, NaMoO4 * 2H2O 100 mg. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with a 0.22 micron filter.

Vitamin Solution (per liter):

[0531] Thiamine hydrochloride 1.0 g, D-(+)-biotin 1.0 g, nicotinic acid 1.0 g, pyridoxine hydrochloride 4.0 g. Each component was dissolved one at a time in Di H2O, pH was adjusted to 3.0 with HCl/NaOH, and then the solution was q.s. to volume and filter sterilized with 0.22 micron filter.

Macro Salt Solution (per liter):

[0532] $MgSO_4 * 7H_2O$ 296 g, citric acid monohydrate 296 g, ferric ammonium citrate 49.6 g. All components were dissolved in water, q.s. to volume and filter sterilized with 0.22 micron filter.

Feed solution (per kilogram):

[0533] Glucose 0.590 kg, Di H_2O 0.393 kg, K_2HPO_4 7.4 g, and 100% Foamblast882 8.9 g. All components were mixed together and autoclaved. After autoclaving the feed solution, nutrient supplements are added to the feed bottle in a sterile hood. Post sterilization additions to the feed are (per kilogram of feed solution), Macro Salt Solution 5.54ml, Vitamin Solution 6.55ml, 1000X Modified Trace Metal Solution 0.82ml.

(ii) Analysis

[0534] Isoprene, Oxygen, Nitrogen, and Carbon Dioxide levels in the off-gas are determined independently by two mass spectrometers, an iSCAN (Hamilton Sundstrand), and a Hiden HPR20 (Hiden Analytical) mass spectrometer.

[0535] Dissolved Oxygen in the fermentation broth is measured by sanitary, sterilizable probe with an optical sensor provided Hamilton Company.

[0536] The citrate, glucose, acetate, and mevalonate concentrations in the fermentor broth is determined in broth samples taken at 4 hour intervals by an HPLC analysis. Concentration in broth samples are determined by comparison of the refractive index response versus a previously generated calibration curve using standard of a known concentration.

CLAIMS

What is claimed is:

1. Recombinant cells capable of producing of isoprene, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprene.

2. The cells of claim 1, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate.

3. The cells of claim 1, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

4. The cells of claim 1, wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.*

5. The cells of claim 1, wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.*

6. The cells of claim 1, the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide.

7. The cells of claim 1, the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula.*

8. The cells of claim 1, the isoprene synthase polypeptide is selected from the group consisting of *Pueraria montana* or *Pueraria lobata, Populus tremuloides, Populus alba, Populus nigra*, and *Populus trichocarpa*.

9. The cells of claim 1, wherein one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

10. The cells of claim 9, the recombinant cells further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides.

11. The cells of claim 1, wherein the one or more nucleic acids is placed under an inducible promoter or a constitutive promoter.

12. The cells of claim 1, wherein the one or more nucleic acids is cloned into one or more multicopy plasmids.

13. The cells of claim 1, wherein the one or more nucleic acids is integrated into a chromosome of the cells.

14. The cells of claim 1, wherein the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells.

15. The cells of claim 1, wherein the recombinant cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae* and *Yarrowia lipolytica*.

16. Recombinant cells capable of producing isoprenoid precursors, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and one or more nucleic acids encoding one or more polypeptides of

the complete MVA pathway, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprenoid precursors.

17. The cells of claim 16, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate.

18. The cells of claim 16, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

19. The cells of claim 16 wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.*

20. The cells of claim 16, wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.*

21. The cells of claim 16, wherein one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

22. The cells of claim 16, wherein the one or more nucleic acids is placed under an inducible promoter or a constitutive promoter.

23. The cells of claim 16, wherein the one or more nucleic acids is cloned into one or more multicopy plasmids.

24. The cells of claim 16, wherein the one or more nucleic acids is integrated into a chromosome of the cells.

25. The cells of claim 16, wherein the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells.

26. The cells of claim 16, wherein the recombinant cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae* and *Yarrowia lipolytica.*

27. Recombinant cells capable of producing of isoprenoids, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and (i) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway and (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein culturing of said recombinant cells in a suitable media provides for the production of isoprenoids.

28. The cells of claim 27, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate.

29. The cells of claim 27, wherein the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

30. The cells of claim 27, wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum, Clostridium acetobutilicum, Nostoc punctiforme, Rhodopseudomonas palustris, Pantoea, Mucilaginibacter paludis, Thermobifida fusca, Bifidobacterium breve, Rahnella aquatili, Bifidobacterium animalis, Gardnerella vaginalis, Streptomyces avermitilis, Lactobacillus plantarum,* and *Lactobacillus reuteri.*

31. The cells of claim 27, wherein the heterologous nucleic acid encoding a polypeptide having phosphoketolase activity is selected from the group consisting of: *Bifidobacterium longum, Enterococcus galliniarum,* and *Clostridium acetobutilicum.*

32. The cells of claim 27, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene.

33. The cells of claim 27, the isoprenoid is a sesquiterpene.

34. The cells of claim 27, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-famesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

35. The cells of claim 27, wherein one or more polypeptides of the complete MVA pathway is selected from (a) an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA; (b) an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA (e.g., HMG synthase); (c) an enzyme that converts HMG-CoA to mevalonate; (d) an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate; (e) an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and (f) an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

36. The cells of claim 27, wherein the one or more nucleic acids is placed under an inducible promoter or a constitutive promoter.

37. The cells of claim 27, wherein the one or more nucleic acids is cloned into one or more multicopy plasmids.

38. The cells of claim 27, wherein the one or more nucleic acids is integrated into a chromosome of the cells.

39. The cells of claim 1, wherein the recombinant host cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells.

40. The cells of claim 1, wherein the recombinant host cells are selected from the group consisting of *Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces*

griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisieae and *Yarrowia lipolytica.*

41. A method of producing isoprene comprising: (a) culturing the recombinant cell of claim 1 under conditions suitable for producing isoprene and (b) producing isoprene.

42. A methods of producing an isoprenoid precursor comprising: (a) culturing the recombinant cell of claim 16 under conditions suitable for producing an isoprenoid precursor and (b) producing an isoprenoid precursor.

43. A methods of producing an isoprenoid comprising: (a) culturing the recombinant cell of claim 27 under conditions suitable for producing an isoprenoid and (b) producing an isoprenoid.