PHOSPHOKETOLASES FOR IMPROVED PRODUCTION OF ACETYL COENZYME A-DERIVED METABOLITES, ISOPRENE, ISOPRENOID PRECURSORS, AND ISOPRENOIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/810,696, filed April 10, 2013, and U.S. Provisional Patent Application No.61/834,359, filed June 12, 2013, the disclosures of each of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] This present invention relates to cultured recombinant cells comprising a heterologous phosphoketolase (PKL) polypeptide that are capable of increased production of acetyl coenzyme A-derived metabolites, as well as methods for producing and using the same. In some embodiments, the recombinant cells further comprise one or more mevalonate (MVA) pathway polypeptides for the production of isoprenoid precursors, isoprene and isoprenoids.

BACKGROUND OF THE INVENTION

[0003] Glycolysis allows the metabolic conversion of a carbon source into intermediate compounds such as acetyl-Coenzyme A (acetyl-CoA) which is an important intermediate in the synthesis of essential biological compounds, including polyketides, fatty acids, amino acids, vitamins, isoprene, isoprenoids, phenolics, and alkaloids. Several of these acetyl-CoA derived metabolites have industrial utility. For example, 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.

[0004] Isoprenoids are also acetyl-CoA-derived metabolites that demonstrate industrial utility. For example, isoprenoids are used in pharmaceutical products and as biofuels, food additives, and other specialty chemicals. 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.

[0005] 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.

[0006] For example, 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 acetyl-CoA-derived metabolites, 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.

[0007] What is needed, therefore, are recombinant cells that utilize 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 in the production of isoprene, isoprenoid precursor molecules, and isoprenoids.

[0008] The invention described herein addresses these problems and provides additional benefits as well.

[0009] 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

[0010] The invention provided herein discloses, *inter alia,* cultured recombinant cells, compositions of these cells and methods of using these cells to increase production of metabolic intermediates such as erythrose 4-phosphate (E4P) , glyceraldehyde 3-phosphate (GAP), and acetyl-phosphate (Ac-P) as well as to increase production of isoprenoid precursors, isoprene, isoprenoids, and/or molecules derived from Acetyl-CoA such as amino acids.

[0011] Accordingly, in one aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:1.

[0012] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:2.

[0013] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:3.

[0014] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:4.

[0015] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:5.

[0016] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:6.

[0017] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:7.

[0018] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8.

[0019] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:9.

[0020] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:10.

[0021] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11.

[0022] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:12.

[0023] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:13.

[0024] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:14.

[0025] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:15.

[0026] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:16

[0027] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:17.

[0028] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:18.

[0029] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:19.

[0030] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:20.

[0031] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:21.

[0032] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:22.

[0033] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:23.

[0034] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:24.

[0035] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:25.

[0036] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:26.

[0037] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:27.

[0038] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:28.

[0039] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:29.

[0040] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:30.

[0041] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:31.

[0042] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:32.

[0043] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:33.

[0044] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:34.

[0045] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:35.

[0046] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:36.

[0047] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:37.

[0048] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:38.

[0049] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:39.

[0050] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:40.

[0051] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:41.

[0052] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:42.

[0053] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:43.

[0054] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:44.

[0055] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:45.

[0056] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a

heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:46.

[0057] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:47.

[0058] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:48.

[0059] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:49.

[0060] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:50.

[0061] In another aspect, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:51.

[0062] In some aspects, in any of the embodiments above and/or herein, culturing of the recombinant cell in a suitable media increases one or more of an intracellular amount of erythrose 4-phosphate, an intracellular amount of glyceraldehyde 3-phosphate, or intracellular amount phosphate. In other aspects, in any of the embodiments above and/or herein, the polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In other aspects, in any of the

embodiments above and/or herein, the polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

[0063] In other aspects, provided herein is a recombinant cell disclosed in any of the embodiments above and/or herein capable of producing isoprene, wherein the recombinant cell further comprises (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 the recombinant cell in a suitable media provides for the production of isoprene. In another aspect of the cells disclosed in any of the embodiments above and/or herein, the 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 aspect of the cells disclosed in any of the embodiments above and/or herein, the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide. In another aspect of the cells disclosed in any of the embodiments above and/or herein, the plant isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula*. In another aspect of the cells disclosed in any of the embodiments above and/or 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 of the cells disclosed in any of the embodiments above and/or herein, the recombinant cells further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides.

[0064] In other aspects, provided herein is a recombinant cell disclosed in any of the embodiments above and/or herein capable of producing isoprenoid precursors, wherein the recombinant cell further comprises one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein culturing of the recombinant cell in a suitable media provides for the production of isoprenoid precursors.

[0065] In other aspects, provided herein is a recombinant cell disclosed in any of the embodiments above and/or herein capable of producing isoprenoids, wherein the recombinant cell further comprises (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 the recombinant cell in a suitable media provides for the production of isoprenoids.

[0066] In other aspects, provided herein is a recombinant cell capable of producing an acetyl CoA-derived metabolite, wherein culturing of the recombinant cells disclosed in any of the embodiments above and/or herein in a suitable media provides for the production of the acetyl CoA-derived metabolite.

[0067] In some aspects, in any of the embodiments above and/or herein, the nucleic acid is placed under an inducible promoter or a constitutive promoter. In other aspects of any of the embodiments above and/or herein, the nucleic acid is cloned into one or more multicopy plasmids. In other aspects of any of the embodiments above and/or herein, the nucleic acid is integrated into a chromosome of the cells.

[0068] In other aspects of any of the embodiments above and/or herein, the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells. In other aspects of any of the embodiments above and/or herein, the recombinant cells are selected from the group consisting of *Corynebacteria spp. (e.g., C. glutamicum), Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisiae* and *Yarrowia lipolytica.*

[0069] In other aspects of any of the embodiments above and/or herein, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene. In other aspects of any of the embodiments above and/or herein, the isoprenoid is a sesquiterpene. In other aspects of any of the embodiments above and/or herein, 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.

[0070] In other aspects of any of the embodiments above and/or herein, the acetyl CoA-derived metabolite is selected from the group consisting of polyketides, polyhydroxybutyrate, fatty alcohols, and fatty acids. In other aspects of any of the embodiments above and/or herein, the acetyl CoA-derived metabolite is selected from the group consisting of glutamic acid, glutamine, aspartate, asparagine, proline, arginine, methionine, threonine, cysteine, succinate, lysine, leucine, and isoleucine. In other aspects of any of the embodiments above and/or herein, the acetyl CoA-derived metabolite is selected from the group consisting of acetone, isopropanol, isobutene, and propene.

[0071] In other aspects of any of the embodiments above and/or herein, the suitable media comprises a carbon source. In other aspects of any of the embodiments above and/or herein, the carbon source is a carbohydrate selected from the group consisting of monosaccharide, disaccharide, oligosaccharide, polysaccharide, C6 sugar, C5 sugar, and invert sugar.

[0072] In other aspects, provided herein is a method of producing isoprene comprising: (a) culturing the recombinant cell disclosed in any of the embodiments above and/or herein under conditions suitable for producing isoprene and (b) producing isoprene.

[0073] In other aspects, provided herein is a method of producing an isoprenoid precursor comprising: (a) culturing the recombinant cell disclosed in any of the embodiments above and/or herein under conditions suitable for producing an isoprenoid precursor and (b) producing an isoprenoid precursor.

[0074] In other aspects, provided herein is a method of producing an isoprenoid comprising: (a) culturing the recombinant cell disclosed in any of the embodiments above and/or herein under conditions suitable for producing an isoprenoid and (b) producing an isoprenoid.

[0075] In other aspects, provided herein are methods of producing an acetyl CoA-derived metabolite comprising: (a) culturing the recombinant cell disclosed in any of the embodiments above and/or herein under conditions suitable for producing an acetyl CoA-derived metabolite and (b) producing an acetyl CoA-derived metabolite.

[0076] In other aspects, provided herein are methods for detecting *in vivo* phosphoketolase activity of a polypeptide in a recombinant cell comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid encoding said polypeptide wherein the recombinant cell

is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) detecting *in vivo* phosphoketolase activity of said polypeptide based upon the presence of cell growth.

[0077] In other aspects, provided herein is isolated polypeptides with phosphoketolase activity produced by any methods of screening, identifying, and/or detecting disclosed herein.

[0078] In other aspects, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8. In other aspects, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In other aspects, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to

SEQ ID NO:26. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31.

[0079] In another aspect, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11. In another aspect, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In another aspect, provided herein are recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments of any of the aspects described above or herein, the polypeptide

comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments of any of the aspects described above or herein, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46.

[0080] In some embodiments of any of the aspects described above or herein, culturing of the recombinant cell in a suitable media increases one or more of an intracellular amount of erythrose 4-phosphate, an intracellular amount of glyceraldehyde 3-phosphate, or intracellular amount of acetyl phosphate. In some embodiments of any of the aspects described above or herein, the polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate. In some embodiments of any of the aspects described above or herein, the polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

[0081] In other embodiments of any of the aspects described above or herein, the 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.

[0082] In other aspects, provided herein are recombinant cells capable of producing isoprene, wherein the recombinant cell (such as any recombinant cell provided herein) further comprises a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein culturing of the recombinant cell in a suitable media provides for the production of isoprene with a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) isoprene yield or (b) isoprene specific productivity. In some embodiments of any of the aspects described above or herein, the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide. In some embodiments of any of the aspects described above or herein, the plant isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula.* In some embodiments of any of the aspects described above or herein, the isoprene synthase polypeptide is selected from the group consisting of *Pueraria montana, Pueraria lobata, Populus tremuloides, Populus alba, Populus nigra,* and *Populus trichocarpa*. In some embodiments of any of the aspects described above or herein, the recombinant cells further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides.

[0083] In other aspects, provided herein are recombinant cells capable of producing isoprenoid precursors, wherein the recombinant cell (such as any recombinant cell provided herein) is cultured in a suitable media and produces said isoprenoid precursors.

[0084] In other aspects, provided herein are recombinant cells of producing isoprenoids, wherein the recombinant cell (such as any recombinant cell provided herein) further comprises a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein culturing of the recombinant cell in a suitable media provides for the production of isoprenoids.

[0085] In yet other aspects, provided herein are recombinant cells capable of producing an acetyl CoA-derived metabolite, wherein culturing of the recombinant cell (such as any

recombinant cell provided herein) in a suitable media provides for the production of the acetyl CoA-derived metabolite.

[0086] In some embodiments of any of the aspects described above or herein, the nucleic acid is placed under an inducible promoter or a constitutive promoter. In some embodiments of any of the aspects described above or herein, the nucleic acid is cloned into one or more multicopy plasmids. In some embodiments of any of the aspects described above or herein, the nucleic acid is integrated into a chromosome of the cells.

[0087] In some embodiments of any of the aspects described above or herein, the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells. In some embodiments of any of the aspects described above or herein, the recombinant cells are selected from the group consisting of *Corynebacteria, Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisiae* and *Yarrowia lipolytica.*

[0088] In some embodiments of any of the aspects described above or herein, the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene. In some embodiments of any of the aspects described above or herein, the isoprenoid is a sesquiterpene. In some embodiments of any of the aspects described above or herein, 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.

[0089] In some embodiments of any of the aspects described above or herein, the acetyl CoA-derived metabolite is selected from the group consisting of polyketides, polyhydroxybutyrate, fatty alcohols, and fatty acids. In some embodiments of any of the aspects described above or herein, the acetyl CoA-derived metabolite is selected from the group consisting of glutamic acid, glutamine, aspartate, asparagine, proline, arginine, methionine, threonine, cysteine, succinate, lysine, leucine, and isoleucine. In some embodiments of any of the aspects described above or herein, the acetyl CoA-derived metabolite is selected from the group consisting of acetone, isopropanol, isobutene, and propene.

[0090] In some embodiments of any of the aspects described above or herein, the suitable media comprises a carbon source. In some embodiments of any of the aspects described above or herein, the carbon source is a carbohydrate selected from the group consisting of monosaccharide, disaccharide, oligosaccharide, polysaccharide, C6 sugar, C5 sugar, and invert sugar.

[0091] In other aspects, also provided herein are methods for producing isoprene comprising: (a) culturing the recombinant cell (such as any recombinant cell provided herein) under conditions suitable for producing isoprene and (b) producing isoprene. In other aspects, also provided herein are methods for producing an isoprenoid precursor comprising: (a) culturing the recombinant cell (such as any recombinant cell provided herein) under conditions suitable for producing an isoprenoid precursor and (b) producing an isoprenoid precursor.

[0092] In other aspects, also provided herein are methods for producing an isoprenoid comprising: (a) culturing the recombinant cell (such as any recombinant cell provided herein) under conditions suitable for producing an isoprenoid and (b) producing an isoprenoid.

[0093] In other aspects, also provided herein are methods for producing an acetyl CoA-derived metabolite comprising: (a) culturing the recombinant cell (such as any recombinant cell provided herein) under conditions suitable for producing an acetyl CoA-derived metabolite and (b) producing an acetyl CoA-derived metabolite.

[0094] In other aspects, also provided herein are methods for detecting *in vivo* phosphoketolase activity of a polypeptide in a recombinant cell comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid sequence encoding said polypeptide wherein the recombinant cell is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) detecting in vivo phosphoketolase activity of said polypeptide based upon the presence of cell growth.

[0095] In other aspects, also provided herein are isolated polypeptides with phosphoketolase activity detected by any of the methods described above or herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0096] Figure 1 depicts an engineered metabolic pathway with phosphoketolase (PKL) present. PKLs 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 PKL-catalyzed reaction(s) is subsequently converted to acetyl-CoA for use in the MVA pathway or can be converted to acetate. Other products of PKL-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. Acetyl-Coa can be converted to many products such as polyketides, fatty acids and amino acids such as lysine.

[0097] Figure 2 is a diagram of the center representative sequences of the 22 Clusters of identified PKLs.

[0098] Figure 3 is a diagram of identified phosphoketolases in Cluster 1.

[0099] Figure 4 is a diagram of identified phosphoketolases in Cluster 2.

[0100] Figure 5 is a diagram of identified phosphoketolases in Cluster 3.

[0101] Figure 6 is a diagram of identified phosphoketolases in Cluster 4.

[0102] Figure 7 is a diagram of identified phosphoketolases in Cluster 5.

[0103] Figure 8 is a diagram of identified phosphoketolases in Cluster 6.

[0104] Figure 9 is a diagram of identified phosphoketolases in Cluster 7.

[0105] Figure 10 is a diagram of identified phosphoketolases in Cluster 8.

[0106] Figure 11 is a diagram of identified phosphoketolases in Cluster 9.

[0107] Figure 12 is a diagram of identified phosphoketolases in Cluster 10.

[0108] Figure 13 is a diagram of identified phosphoketolases in Cluster 11.

[0109] Figure 14 is a diagram of identified phosphoketolases in Cluster 12.

[0110] Figure 15 is a diagram of identified phosphoketolases in Cluster 13.

[0111] Figure 16 is a diagram of identified phosphoketolases in Cluster 14.

[0112] Figure 17 is a diagram of identified phosphoketolases in Cluster 15.

[0113] Figure 18 is a diagram of identified phosphoketolases in Cluster 16.

[0114] Figure 19 is a diagram of identified phosphoketolases in Cluster 17.

[0115] Figure 20 is a diagram of identified phosphoketolases in Cluster 18.

[0116] Figure 21 is a diagram of identified phosphoketolases in Cluster 19.

[0117] Figure 22 is a diagram of identified phosphoketolases in Cluster 20.

[0118] Figure 23 is a diagram of identified phosphoketolases in Cluster 21.

[0119] Figure 24 is a diagram of identified phosphoketolases in Cluster 22.

[0120] Figure 25 depicts the plasmid map of pCMP1321, expressing *Enterococcus gallinarum* phosphoketolase.

[0121] Figure 26 depicts the plasmid map of pMCS530, expressing *Bifidobacterium dentium* phosphoketolase.

[0122] Figure 27 depicts the plasmid map of pMCS531, expressing *Bifidobacterium bifidum* phosphoketolase.

[0123] Figure 28 depicts the plasmid map of pMCS532, expressing *Bifidobacterium gallicum* phosphoketolase.

[0124] Figure 29 depicts the plasmid map of pMCS533, expressing *Lactobacillus buchneri* phosphoketolase.

[0125] Figure 30 depicts the plasmid map of pMCS534, expressing *Burkholderia phytofermans* phosphoketolase.

[0126] Figure 31 depicts the plasmid map of pMCS535, expressing *Clostridium acetobutylicum* optimized phosphoketolase.

[0127] Figure 32 is a series of SDS-PAGE coomasie stained gels showing protein expression in strains expressing phosphoketolase. A) soluble protein and B) insoluble protein from cells expressing *B. longum* PKL (lane 1), *E. gallinarum* PKL (lane 2), *C. acetobutylicum* PKL (lane 3), *B. dentium* PKL (lane 4), *B. bifidum* PKL (lane 5), *B. gallicum* PKL (lane 6), *L. buchneri* PKL (lane 7), *B. phytofermans* PKL (lane 8), and *C. acetobutylicum* codon optimized PKL (lane 9).

[0128] Figure 33 is a graph showing *in vitro* activity of *B. longum* PKL, *E. gallinarum* PKL, *C. acetobutylicum* PKL, *B. dentium* PKL, *B. bifidum* PKL, *B. gallicum* PKL, *L. buchneri* PKL, *B. phytofermens* PKL, and *C. acetobutylicum* codon optimized PKL in the presence of F6P substrate as measured by Ac-P yield.

[0129] Figure 34 is a graph showing that the transketolase mutant grew on glucose only with supplement containing six aromatic compounds and pyridoxine.

[0130] Figure 35 is a graph showing that phosphoketolases from *E. gallinarum* and *C. acetobutylicum* restored growth to the transketolase mutant on glucose without supplement.

[0131] Figure 36 is a graph showing that the transketolase mutant did not grow on xylose with or without supplement containing six aromatic compounds and pyridoxine.

[0132] Figure 37 is a graph showing that phosphoketolases from *E. gallinarum* and *C. acetobutylicum* restored growth to the transketolase mutant on xylose without supplement.

[0133] Figure 38 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).

[0134] Figure 39 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.

[0135] Figure 40 depicts the cumulative isoprene yield of various PKL enzymes in an MD891(ackA-)host.

[0136] Figure 41 depicts end of fermentation (EOF) isoprene titer of various PKL enzymes in an MD891(ackA-)host.

[0137] Figure 42 depicts a generic plasmid map of a plasmid suitable for co-expression of PKL in accordance with any of the compositions, cells, or methods disclosed herein.

DETAILED DESCRIPTION

[0138] The invention provided herein discloses, *inter alia,* compositions and methods for the production of acetyl coenzyme A-derived metabolites, 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 acetyl coenzyme A-derived metabolites, 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 acetyl coenzyme A-derived metabolites, 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 acetyl coenzyme A-derived metabolites, 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 acetyl coenzyme A-derived metabolites, 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.

[0139] 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.

[0140] 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 acetyl-CoA-derived metabolites, 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.

[0141] 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 [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.

[0142] 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 (Figure 1). 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.

[0143] 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.

[0144] The present invention is directed to the use of phosphoketolase enzymes in the production of acetyl-CoA-derived metabolites, 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

[0145] The mevalonate-dependent biosynthetic pathway is particularly important for the production of 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 intracellular pools of E4P, GAP, and Ac-P produced by the use of a phosphoketolase polypeptide for the increased biosynthesis of acetyl CoA 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 (Figure 1). Furthermore, the increased biosynthesis of acetyl-CoA can result in the increased synthesis of acetyl-CoA-derived metabolites such as fatty acids, amino acids, and acetone (Figure 1). The increased intracellular amount-CoA production by this alternate PKL pathway is therefore advantageous for commercial applications.

[0146] Acetone is produced by certain microorganisms, such as *Clostridium acetobutylicum.* It starts out with condensation of two molecules of acetyl-CoA into acetoacetyl-CoA by acetyl-CoA acetyltransferase (EC 2.3.1 .9). Acetoacetyl-CoA is then converted into acetoacetate by a reaction with acetic acid or butyric acid resulting in the production of acetyl-CoA or butyryl-CoA. This reaction is catalyzed by an enzyme such as acetoacetylCoA transferase (EC 2.8.3.8). AcetoacetylCoA transferase is known from various organisms, such as E. coli or C. acetobutyiicum. However, also other enzymes can catalyze this reaction, such as 3-oxoacid CoA transferase (EC 2.8.3.5) or succinate CoA ligase (EC 6.2.1 .5). In the last step of the reaction, acetoacetate is converted into acetone by a decarboxylation step catalyzed by acetoacetate decarboxylase (EC 4.1 .1 .4). Acetone can be subsequently converted to isopropanol, isobutene and/or propene as described in WO 2013/07786, the contents of which are expressly incorporated herein by reference in their entirety with respect to acetone, isoprene and propene.

[0147] Accordingly, in certain aspects, the invention provides recombinant cells with an increased intracellular amount of erythrose 4-phosphate, an increased intracellular amount of glyceraldehyde 3-phosphate, and/or an increased intracellular amount phosphate, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having

phosphoketolase activity, and wherein the cells produce the increased intracellular amount of erythrose 4-phosphate, increased intracellular amount of glyceraldehyde 3-phosphate, and/or increased intracellular amount phosphate as compared to cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0148] In some aspects, the invention provides recombinant cells with an increased intracellular amount of acetyl-CoA, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity, and wherein the cells produce the increased intracellular amount of acetyl-CoA as compared to cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0149] 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.

[0150] 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.

[0151] In still other aspects, the present invention provides recombinant cells capable of producing 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.

[0152] In yet other aspects, the present invention provides recombinant cells capable of producing 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.

[0153] In other aspects, the present invention provides recombinant cells capable of producing an acetyl CoA-derived metabolite, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity, wherein the cells are capable of producing recoverable amounts of the acetyl CoA-derived metabolite. In certain embodiments, the present invention provides recombinant cells capable of enhanced production of an acetyl CoA-derived metabolite, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity, wherein the cells produce increased amounts of the acetyl CoA-derived metabolite as compared to acetyl CoA-derived metabolite producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0154] In any of the aspects herein, the present invention provides recombinant cells, wherein the cells can 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.

[0155] In some embodiments, the present invention provides recombinant cells capable of producing 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.

[0156] In some embodiments, the present invention provides recombinant cells capable of producing 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, (ii) a heterologous nucleic acid encoding an polyprenyl pyrophosphate 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 isoprenoids compared to isoprenoid producing cells that do not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

[0157] In other embodiments, the present invention provides recombinant cells capable of enhanced production of an acetyl CoA-derived metabolite, wherein the cells comprise one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity and 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 the acetyl CoA-derived metabolite as compared to acetyl CoA-derived metabolite producing cells that do

not comprise the one or more heterologous nucleic acids encoding a polypeptide having phosphoketolase activity.

General Techniques

[0158] 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

[0159] 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.

[0160] As used herein, the term "polypeptides" includes polypeptides, proteins, peptides, fragments of polypeptides, and fusion polypeptides.

[0161] 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.

[0162] 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.

[0163] 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.

[0164] 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.

[0165] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum* and used to transform an *E. coli* is a heterologous nucleic acid.

[0166] 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.

[0167] 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.

[0168] 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.

[0169] 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.

[0170] 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."

[0171] 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.

[0172] 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).

[0173] 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.

[0174] 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.

[0175] By "titer," it is meant the mass of the product produced by the recombinant cells divided by the volume of the culture.

[0176] 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.

[0177] 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.

[0178] As used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

[0179] 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

[0180] 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 produce mevalonate, isoprenoid precursor molecules, isoprene and/or isoprenoids or can be utilized to produce acetyl-CoA-derived metabolites.

[0181] As used herein, the term "acetyl-CoA-derived metabolite" can refer to a metabolite resulting from the catalytic conversion of acetyl-CoA to said metabolite. The conversion can be a one-step reaction or a multi-step reaction. For example, acetone is an acetyl-CoA derived metabolite that is produced from acetyl-CoA by a three step reaction (*e.g.*, a multi-step reaction): 1) the condensation of two molecules of acetyl-CoA into acetoacetyl-CoA by acetyl-CoA acetyltransferase; 2) conversion of acetoacetyl-CoA into acetoacetate by a reaction with acetic acid or butyric acid resulting in the production of acetyl-CoA or butyryl-CoA; and 3) conversion of acetoacetate into acetone by a decarboxylation step catalyzed by acetoacetate decarboxylase. Acetone can be subsequently converted to isopropanol, isobutene and/or propene which are also expressly contemplated herein to be acetyl-CoA-derived metabolites. In some embodiments, the

acetyl CoA-derived metabolite is selected from the group consisting of polyketides, polyhydroxybutyrate, fatty alcohols, and fatty acids. In some embodiments, the acetyl CoA-derived metabolite is selected from the group consisting of glutamic acid, glutamine, aspartate, asparagine, proline, arginine, methionine, threonine, cysteine, succinate, lysine, leucine, and isoleucine. In some embodiments, the acetyl CoA-derived metabolite is selected from the group consisting of acetone, isopropanol, isobutene, and propene. Thus the amount of these compounds (*e.g.*, acetyl-CoA, acetyl-CoA-derived metabolite, acetyl-P, E4P, etc.) produced from a carbohydrate substrate may be increased.

[0182] Production of acetyl-P and acetyl-CoA 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.

Exemplary phosphoketolase polypeptides and nucleic acids

[0183] 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, Figures 2-24 and Example 2). Additionally, Table 1 and Table 2 provides a non-limiting list of certain exemplary phosphoketolases from different species which may be utilized within embodiments of the invention.

[0184] 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.

[0185] In some instances, phosphoketolases from certain organisms can be selected if the organisms lack a phosphofructokinase gene in its genome.

[0186] In yet another example, phosphoketolases can be selected based on a secondary structure of the amino acid sequence and/or the method described in Example 1.

[0187] In still another example, phosphoketolases can be selected based on an *in vitro* assay as described in Example 6.

[0188] In still another example, phosphoketolases can be selected based on an *in vivo* assay as described in Example 7. In some aspects, provided herein is a method for determining the presence of *in vivo* phosphoketolase activity of a polypeptide comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid sequence encoding said polypeptide wherein the recombinant cell is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) determining the presence of *in vivo* phosphoketolase activity of said polypeptide based upon the amount of observed cell growth. In some aspects, provided herein is a method of identifying a polypeptide with phosphoketolase activity comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid sequence encoding a polypeptide suspected of having phosphoketolase activity wherein the recombinant cell is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) identifying the polypeptide with phosphoketolase activity when cell growth is observed. In some aspects, provided herein is a method for detecting *in vivo* phosphoketolase activity of a polypeptide in a recombinant cell comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid sequence encoding said polypeptide wherein the recombinant cell is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) detecting *in vivo* phosphoketolase activity of said polypeptide based upon the presence of cell growth.

[0189] As provided herein, phosphoketolase activity can improve production of acetyl-CoA-derived metabolites, isoprenoid precursors (e.g., IPP), 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 acetyl-CoA-derived metabolites, isoprenoid precursors (e.g., IPP), isoprene, and/or isoprenoids.

[0190] 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 (*e.g.*, growth). As used herein, "performance index" refers to calculated activity per unit relative to a parental molecule. In some aspects of any of the embodiments disclosed herein, the parental molecule used in the calculation of the performance index is a phosphoketolase from *E. gallinarum.* In some embodiments, the parental molecule has a performance index of one, by definition. In other embodiments, a performance index greater than one (PI >1.0) indicates improved activity of a phosphoketolase compared to the parent molecule (*e.g.*, a phosphoketolase from *E. gallinarum).*

[0191] 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%.

[0192] 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.

[0193] 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.

[0194] 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.

[0195] In other embodiments, the performance index values for properties of interest, including but not limited to, (a) cell growth on glucose, (b) cell growth on xylose, (c) cell growth

on glucose-6-phosphate or (d) production of intracellular Acetyl-phosphate for a recombinant cell comprising a polypeptide having phosphoketolase activity as set forth herein and one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway is greater than 1, such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*).

[0196] In other embodiments, the performance index values for properties of interest, including but not limited to, (a) protein solubility, (b) protein expression, or (c) F6P specific activity for a polypeptide having phosphoketolase activity in a recombinant cell further comprising one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway is greater than 1, such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*).

[0197] In other embodiments, the performance index values for properties of interest, including but not limited to, (a) isoprene yield protein solubility or (b) isoprene specific productivity for a recombinant cell comprising (i) 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 is greater than 1, such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.*, a phosphoketolase from *E. gallinarum*).

[0198] 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0199] 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 aspects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In other aspects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp.,* and/or *Neosartorya fischeri*. In other aspects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In yet other aspects, exemplary phosphoketolase nucleic acids include, for example, a phosphoketolase isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*.

[0200] 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*. Additional phosphoketolases that can be used, include but are not limited to, *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and *Clostridium acetobutylicum*.

[0201] 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, the 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.

[0202] In any of the embodiments 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 any of the phosphoketolase nucleic acid sequences described herein. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycobacterium gilvum* 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:52. In some embodiments, the phosphoketolase nucleic acid encoded by the *Shewanella baltica* 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:53. In some embodiments, the phosphoketolase nucleic acid encoded by the *Lactobacillus rhamnosus* 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:54. In some embodiments, the phosphoketolase nucleic acid encoded by the *Lactobacillus crispatus* 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:55. In some embodiments, the phosphoketolase nucleic acid encoded by the *Leuconostoc citreum* 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:56. In some embodiments, the phosphoketolase nucleic acid encoded by the *Bradyrhizobium 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:57. In some

embodiments, the phosphoketolase nucleic acid encoded by the *Brucella microti* 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:58. In some embodiments, the phosphoketolase nucleic acid encoded by the *Lactobacillus salivarius* 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:59. In some embodiments, the phosphoketolase nucleic acid encoded by the *Rhodococcus imtechensis* 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:60. In some embodiments, the phosphoketolase nucleic acid encoded by the *Burkholderia xenovorans* 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:61. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycobacterium intracellulare* 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:62. In some embodiments, the phosphoketolase nucleic acid encoded by the *Nitrosomonas 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:63. In some embodiments, the phosphoketolase nucleic acid encoded by the *Schizosaccharomyces pombe* 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:64. In some embodiments, the phosphoketolase nucleic acid encoded by the *Lactobacillus buchneri* 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:65. In some embodiments, the phosphoketolase nucleic acid encoded by the *Streptomyces ghanaensis* 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:66. In some embodiments, the phosphoketolase nucleic acid encoded by the *Cyanothece 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:67. In some embodiments, the phosphoketolase nucleic acid encoded by the *Neosartorya fischeri* 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:68. In some embodiments, the phosphoketolase nucleic acid encoded by the *Enterococcus faecium* 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:69. In some embodiments, the phosphoketolase nucleic acid encoded by the *Listeria grayi* 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:70. In some embodiments, the phosphoketolase nucleic acid encoded by the *Enterococcus casseliflavus* 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:71. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma alligatoris* 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:72. In some embodiments, the phosphoketolase nucleic acid encoded by the *Carnobacterium 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:73. In some embodiments, the phosphoketolase nucleic acid encoded by the *Melissococcus plutonius* 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 any one of SEQ ID NOs:74 and 76. In some embodiments, the phosphoketolase nucleic acid encoded by the *Tetragenococcus halophilus* 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:75. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma arthritidis* 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:77. In some embodiments, the phosphoketolase nucleic acid encoded by the *Streptococcus agalactiae* 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:78. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma agalactiae* 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:79. In some embodiments, the phosphoketolase nucleic acid encoded by the *Streptococcus gordonii* 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:80. In some embodiments, the phosphoketolase nucleic acid encoded by the *Kingella oralis* 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:81. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma fermentans* 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:82. In some embodiments, the phosphoketolase nucleic acid encoded by the *Granulicatella adiacens* 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:83. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma hominis* 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:84. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma crocodyli* 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:85. In some embodiments, the phosphoketolase nucleic acid encoded by the *Neisseria 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:86. In some embodiments, the phosphoketolase nucleic acid encoded by the *Eremococcus coleocola* 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:87. In some embodiments, the phosphoketolase nucleic acid encoded by the *Aerococcus urinae* 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:88. In some embodiments, the phosphoketolase nucleic acid encoded by the *Kingella kingae* 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:89. In some embodiments, the phosphoketolase nucleic acid encoded by the *Streptococcus criceti* 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 any one of SEQ ID NOs:90 and 91. In some embodiments, the phosphoketolase nucleic acid encoded by the *Mycoplasma*

columbinum 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:92.

[0203] 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycobacterium gilvum* phosphoketolase amino 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Shewanella baltica* phosphoketolase amino acid sequence SEQ ID NO:2. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus rhamnosus* phosphoketolase amino 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus crispatus* phosphoketolase amino acid sequence SEQ ID NO:4. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium longum* phosphoketolase amino acid sequence SEQ ID NO:5. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Leuconostoc citreum* phosphoketolase amino acid sequence SEQ ID NO:6. 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%, 85%, 80%, 75%, 70%, 65%, or 60% sequence identity to the phosphoketolase polypeptide encoded by the *Bradyrhizobium sp.* phosphoketolase amino acid sequence SEQ ID NO:7. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Enterococcus faecium* phosphoketolase amino acid sequence SEQ ID NO:8. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Brucella microti* phosphoketolase amino acid sequence SEQ ID NO:9. 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%, 85%, 80%, 75%, 70%, 65%, or 60% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus salivarius* phosphoketolase amino acid sequence SEQ ID NO:10. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Streptococcus agalactiae* phosphoketolase amino acid sequence SEQ ID NO:11. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Rhodococcus imtechensis* phosphoketolase amino acid sequence SEQ ID NO:12. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Burkholderia xenovorans* phosphoketolase amino acid sequence SEQ ID NO:13. 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%, 85%, 80%, 75%, 70%, 65%, or 60% sequence identity to the phosphoketolase polypeptide encoded by the *Mycobacterium intracellulare* phosphoketolase amino acid sequence SEQ ID NO:14. 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%, 85%, 80%, 75%, 70%, 65%, or 60% sequence identity to the phosphoketolase polypeptide encoded by the *Nitrosomonas sp.* phosphoketolase amino acid sequence SEQ ID NO:15. 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%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% sequence identity to the phosphoketolase polypeptide encoded by the *Schizosaccharomyces pombe* phosphoketolase amino acid sequence SEQ ID NO:16. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Leuconostoc mesenteroides* phosphoketolase

amino 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%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% sequence identity to the phosphoketolase polypeptide encoded by the *Streptomyces sp.* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus buchneri* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% sequence identity to the phosphoketolase polypeptide encoded by the *Streptomyces ghanaensis* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, 65%, or 60% sequence identity to the phosphoketolase polypeptide encoded by the *Cyanothece sp.* phosphoketolase amino 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Neosartorya fischeri* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Enterococcus faecium* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Listeria grayi* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Enterococcus casseliflavus* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65%

sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma alligatoris* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Carnobacterium sp.* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Melissococcus plutonius* phosphoketolase amino 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Tetragenococcus halophilus* phosphoketolase amino acid sequence SEQ ID NO:29. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Melissococcus plutonius* phosphoketolase amino acid sequence SEQ ID NO:30. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma arthritidis* phosphoketolase amino acid sequence SEQ ID NO:31. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Streptococcus agalactiae* phosphoketolase amino acid sequence SEQ ID NO:32. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma agalactiae* phosphoketolase amino acid sequence SEQ ID NO:33. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Streptococcus gordonii* phosphoketolase amino acid sequence SEQ ID NO:34. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Kingella oralis* phosphoketolase amino acid sequence SEQ ID NO:35. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma fermentans* phosphoketolase amino acid sequence SEQ ID NO:36. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Granulicatella adiacens* phosphoketolase amino acid sequence SEQ ID NO:37. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma hominis* phosphoketolase amino acid sequence SEQ ID NO:38. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma crocodyli* phosphoketolase amino acid sequence SEQ ID NO:39. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Neisseria sp.* phosphoketolase amino acid sequence SEQ ID NO:40. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Eremococcus coleocola* phosphoketolase amino acid sequence SEQ ID NO:41. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Aerococcus urinae* phosphoketolase amino acid sequence SEQ ID NO:42. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Kingella kingae* phosphoketolase amino acid sequence SEQ ID NO:43. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Streptococcus criceti* phosphoketolase amino acid sequence SEQ ID NO:44. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Streptococcus criceti* phosphoketolase amino acid sequence SEQ ID NO:45. 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%, 85%, 80%, 75%, 70%, or 65% sequence identity to the phosphoketolase polypeptide encoded by the *Mycoplasma columbinum* phosphoketolase amino acid sequence SEQ ID NO:46. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Burkholderia phytofirmans* phosphoketolase amino acid sequence SEQ ID NO:47. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Lactobacillus buchneri* phosphoketolase amino acid sequence SEQ ID NO:48. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium gallicum* phosphoketolase amino acid sequence SEQ ID NO:49. In some embodiments, the phosphoketolase polypeptide can have at least about99%, 98%, 97%, 96%, 95%, 95%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium dentium* phosphoketolase amino acid sequence SEQ ID NO:50. 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%, 85%, 80%, 75%, or 70% sequence identity to the phosphoketolase polypeptide encoded by the *Bifidobacterium bifidum* phosphoketolase amino acid sequence SEQ ID NO:51.

[0204] 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.

[0205] In some aspects, provided herein is a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity as described herein. In some embodiments, the polypeptide having phosphoketolase activity is isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In other aspects, the polypeptide having phosphoketolase activity isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri*. In other aspects, the polypeptide having phosphoketolase activity isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In yet other aspects, the polypeptide having phosphoketolase activity isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*.

[0206] In any of the embodiments herein, 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*).

Methods of using recombinant cells to produce increased amounts of acetyl-CoA and acetyl-derived metabolites

[0207] Also provided herein are methods for the production of acetyl-CoA. In some aspects, the method for producing acetyl-CoA comprises: (a) culturing a composition comprising recombinant cells which have been engineered to increase carbon flux through the phosphoketolase pathway as described herein (including any of the recombinant cells described above), or progeny thereof, capable of producing acetyl-CoA; and (b) producing mevalonate. In some aspects, the method of producing acetyl-CoA comprises the steps of culturing any of the recombinant cells described herein under conditions suitable for the production of acetyl-CoA and allowing the recombinant cells to produce acetyl-CoA. In some aspects, the method of producing acetyl-CoA further comprises a step of recovering the acetyl-CoA.

[0208] As described herein, the methods of producing acetyl-CoA 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; and (b) producing acetyl-CoA. In certain embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid

encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase identified from an *in vivo* screening assay as described in Example 7. Additionally, the recombinant cells can produce acetyl-CoA in concentrations greater than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, *Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, *Mycoplasma arthritidis, Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*, 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 described herein is a heterologous nucleic acid that is integrated into the host cell's chromosome.

[0209] Also provided herein are methods for the production of acetyl-CoA-derived metabolites. In some aspects, the method for producing acetyl-CoA-derived metabolites comprises: (a) culturing a composition comprising recombinant cells which have been engineered to increase carbon flux through the phosphoketolase pathway as described herein (including any of the recombinant cells described above), or progeny thereof, capable of producing acetyl-CoA-derived metabolites; and (b) producing mevalonate. In some aspects, the method of producing acetyl-CoA-derived metabolites comprises the steps of culturing any of the recombinant cells described herein under conditions suitable for the production of acetyl-CoA-derived metabolites and allowing the recombinant cells to produce acetyl-CoA-derived metabolites. In some aspects, the method of producing acetyl-CoA further comprises a step of recovering the acetyl-CoA-derived metabolites.

[0210] As described herein, the methods of producing acetyl-CoA-derived metabolites 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; and (b) producing acetyl-CoA-derived metabolites. In certain embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus*

casseliflavus, *Mycoplasma alligatoris*, *Carnobacterium sp.*, *Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase identified from an *in vivo* screening assay as described in Example 7. Additionally, the recombinant cells can produce acetyl-CoA-derived metabolites in concentrations greater than that of the same cells lacking one or more heterologous copies of a gene encoding an phosphoketolase polypeptide from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, *Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp.*, *Melissococcus plutonius*, *Tetragenococcus halophilus*, *Mycoplasma arthritidis, Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*, 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 described herein is a heterologous nucleic acid that is integrated into the host cell's chromosome.

[0211] In any of the embodiments herein, the acetyl-CoA-derived metabolite can be one or more of polyketides, polyhydroxybutyrate, fatty alcohols, or fatty acids. In any of the embodiments herein, the acetyl-CoA-derived metabolite can be one or more of an amino acid selected from the group consisting of: glutamic acid, glutamine, aspartate, asparagine, proline, arginine, methionine, threonine, cysteine, lysine, leucine, and isoleucine. In some embodiments, the acetyl-CoA-derived metabolite is succinate. In any of the embodiments herein, the acetyl-CoA-derived metabolite can be one or more of acetone, isopropanol, isobutene, or propene.

[0212] Also provided herein are methods for producing acetyl-CoA-derived metabolites comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said acetyl-CoA-derived metabolites. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4,

greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum.* 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.

[0213] Additionally provided herein are methods for producing acetyl-CoA-derived metabolites comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or

(c) fructose-6-phosphate (F6P) Specific Activity and producing said acetyl-CoA-derived metabolites. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of

about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0214] Further provided herein are methods for producing acetyl-CoA-derived metabolites comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said acetyl-CoA-derived metabolites. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any

of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0215] Provided herein are methods for producing acetyl-CoA-derived metabolites comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more

of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing said acetyl-CoA-derived metabolites. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to

0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

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

[0216] 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.

[0217] 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.

[0218] 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.

Upper MVA pathway polypeptides

[0219] 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 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.

[0220] 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.

[0221] 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.

[0222] 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

[0223] 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.

[0224] 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

[0225] 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.

[0226] 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 cannot 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).

[0227] 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.

[0228] 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.

[0229] 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.

[0230] 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.

[0231] 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:95. 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:96. 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:97. 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:98. 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).

[0232] 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

[0233] 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.

[0234] 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.

[0235] 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.

[0236] 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.

[0237] 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:99. 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:100. 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:101. 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:102. 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 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).

[0238] 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

[0239] 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.

[0240] 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:103 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.

[0241] In one embodiment, the gene encoding a protein having the amino acid sequence of SEQ ID NO:103 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.

[0242] 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:103 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:103 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:103, which is calculated by performing alignment of the amino acid sequence of SEQ ID NO:103 and the different amino acid sequence with the use of a program for searching for a sequence similarity.

[0243] 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:103 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.

[0244] 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:103 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 °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).

[0245] 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:103 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:103 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.

[0246] The activity of an acetoacetyl-CoA synthase having an amino acid sequence that differs from the amino acid sequence of SEQ ID NO:103 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

[0247] 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.

[0248] 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.

[0249] In certain aspects, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma*

hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti, and/or *Mycoplasma columbinum*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24.

[0250] 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.

[0251] 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*).

[0252] 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.

[0253] 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.

[0254] 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 comparison 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 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*).

[0255] 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.

[0256] 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 comparison 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 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, 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*).

[0257] 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.

[0258] 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 comparison 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 increase 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*).

[0259] 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.

[0260] 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 comparison 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.

[0261] Also provided herein are mevalonate-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein

said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about

8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0262] Additionally provided herein are mevalonate-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater

than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0263] Further provided herein are mevalonate-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ

ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or

more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0264] Provided herein are mevalonate-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8,

greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

Methods of using recombinant cells to produce increased amounts of mevalonate

[0265] 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 through the phosphoketolase pathway 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.

[0266] 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 embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24. 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum* 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma*

crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti, and/or *Mycoplasma columbinum* is a heterologous nucleic acid that is integrated into the host cell's chromosome.

[0267] 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.

[0268] 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 recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or*

Neosartorya fischeri. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from an organism listed in Table 1, Table 2 and/or Figures 3-24.

[0269] 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.

[0270] 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 recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans,*

Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from an organism listed in Table 1, Table 2 and/or Figures 3-24.

[0271] 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.

[0272] 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.

[0273] 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 recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus*

criceti, and/or *Mycoplasma columbinum*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from an organism listed in Table 1, Table 2 and/or Figures 3-24.

[0274] 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 certain embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp.,*

Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti, and/or *Mycoplasma columbinum*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from an organism listed in Table 1, Table 2 and/or Figures 3-24.

[0275] 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.

[0276] 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 recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri*. In some embodiments, the recombinant cells

comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from an organism listed in Table 1, Table 2 and/or Figures 3-24. In some aspects, the method of producing mevalonate further comprises a step of recovering the mevalonate.

[0277] Also provided herein are methods for producing mevalonate comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said mevalonate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ

ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0278] Additionally provided herein are methods for producing mevalonate comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or

more polypeptides of the upper MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing said mevalonate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about

8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0279] Further provided herein are methods for producing mevalonate comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the upper MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said mevalonate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the

polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0280] Provided herein are methods for producing mevalonate comprising culturing a recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more

polypeptides of the upper MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing said mevalonate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

Recombinant Cells Capable of Producing Isoprene

[0281] 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.

[0282] 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 E4P, GAP, Ac-P, and/or acetyl-CoA in recombinant cells comprising the mevalonate pathway 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.

[0283] As described herein, the present invention provides recombinant cells capable of producing 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.

[0284] 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 and subsequent isoprenoid precursor, isoprenoid, and/or isoprene production. The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flux through the phosphoketolase pathway for production of acetyl-CoA that can be used for mevalonate production and subsequent isoprenoid precursor, isoprenoid, and/or isoprene production. 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 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

[0285] 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.

[0286] 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.

[0287] 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.*

[0288] 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.

[0289] 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.

[0290] 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.

[0291] 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.

[0292] 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

[0293] 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 through the phosphoketolase 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.*

[0294] 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.

[0295] 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.

[0296] 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.

[0297] 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.

[0298] 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.

[0299] 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.

[0300] 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*).

[0301] 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.

[0302] 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.

[0303] 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).

[0304] 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.

[0305] 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.

[0306] 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.

Isoprene biosynthetic pathway

[0307] Isoprene can be produced from two different alcohols, 3-methyl-2-buten-1-ol and 2-methyl-3-buten-2-ol. For example, in a two-step isoprene biosynthetic pathway, dimethylallyl diphosphate is converted to 2-methyl-3-buten-2-ol by an enzyme such as a synthase (*e.g.*, a 2-methyl-3-buten-2-ol synthase), followed by conversion of 2-methyl-3-buten-2-ol to isoprene by a 2-methyl-3-buten-2-ol dehydratase. As another example, in a three-step isoprene biosynthetic pathway, dimethylallyl diphosphate is converted to 3-methyl-2-buten-1-ol by either a phosphatase or a synthase (*e.g.*, a geraniol synthase or farnesol synthase) capable of converting dimethylallyl diphosphate to 3-methyl-2-buten-1-ol, 3-methyl-2-buten-1-ol is converted to 2-methyl-3-buten-2-ol by a 2-methyl-3-buten-2-ol isomerase, and 2-methyl-3-buten-2-ol is converted to isoprene by a 2-methyl-3-buten-2-ol dehydratase. *See* for example, U.S. Patent Application Publication No.: US 20130309742 A1 and U.S. Patent Application Publication No.: US 20130309741 A1.

[0308] In some aspects of the invention, the cells described in any of the compositions or methods described herein (including host cells that have been modified as described herein) further comprise one or more nucleic acids encoding a polypeptide of an isoprene biosynthetic pathway selected from the group consisting of 2-methyl-3-buten-2-ol dehydratase, 2-methyl-3-butene-2-ol isomerase, and 3-methyl-2-buten-1-ol synthase. In some aspects, the polypeptide of an isoprene biosynthetic pathway is an endogenous polypeptide. In some aspects, the

endogenous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a constitutive promoter. In some aspects, the endogenous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to an inducible promoter. In some aspects, the endogenous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a strong promoter. In a particular aspect, the cells are engineered to overexpress the endogenous polypeptide of an isoprene biosynthetic pathway relative to wild-type cells. In some aspects, the endogenous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a weak promoter.

[0309] In some aspects, the polypeptide of an isoprene biosynthetic pathway is a heterologous polypeptide. In some aspects, the cells comprise more than one copy of a heterologous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway. In some aspects, the heterologous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a constitutive promoter. In some aspects, the heterologous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to an inducible promoter. In some aspects, the heterologous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a strong promoter. In some aspects, the heterologous nucleic acid encoding a polypeptide of an isoprene biosynthetic pathway is operably linked to a weak promoter.

[0310] The nucleic acids encoding a polypeptide(s) of an isoprene biosynthetic pathway can be integrated into a genome of the host cells or can be stably expressed in the cells. The nucleic acids encoding a polypeptide(s) of an isoprene biosynthetic pathway can additionally be on a vector.

[0311] Exemplary nucleic acids encoding a polypeptide(s) of an isoprene biosynthetic pathway include nucleic acids that encode a polypeptide, fragment of a polypeptide, peptide, or fusion polypeptide that has at least one activity of a polypeptide of an isoprene biosynthetic pathway such as a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide. Exemplary polypeptide(s) of an isoprene biosynthetic pathway and nucleic acids encoding polypeptide(s) of an isoprene biosynthetic pathway include naturally-occurring polypeptides and nucleic acids from any of the source organisms described herein. In addition, variants of polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-

3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide) can possess improved activity such as improved enzymatic activity.

[0312] In some aspects, a polypeptide of an isoprene biosynthetic pathway is a phosphatase. Exemplary phosphatases include a phosphatase from *Bacillus subtilis* or *Escherichia coli*. In some embodiments, the phosphatase is a 3-methyl-2-buten-1-ol synthase polypeptide or variant thereof. In some aspects, a polypeptide of an isoprene biosynthetic pathway is a terpene synthase (*e.g.*, a geraniol synthase, farnesol synthase, linalool synthase or nerolidol synthase). Exemplary terpene synthases include a terpene synthase from *Ocimum basilicum, Perilla citriodora, Perilla frutescans*, *Cinnamomom tenuipile, Zea mays* or *Oryza sativa.* Additional exemplary terpene synthases include a terpene synthase from *Clarkia breweri*, *Arabidopsis thaliana*, *Perilla setoyensis*, *Perilla frutescens*, *Actinidia arguta*, *Actinidia polygama*, *Artemesia annua*, *Ocimum basilicum*, *Mentha aquatica*, *Solanum lycopersicum*, *Medicago trunculata*, *Populus trichocarpa*, *Fragaria vesca*, or *Fragraria ananassa*. In some embodiments, the terpene synthase is a 3-methyl-2-buten-1-ol synthase polypeptide or variant thereof. For example, a terpene synthase described herein can catalyze the conversion of dimethylallyl diphosphate to 3-methyl-2-buten-1-ol (*e.g.*, a 3-methyl-2-buten-1-ol synthase). In some aspects, a terpene synthase described herein can catalyze the conversion of dimethylallyl diphosphate to 2-methyl-3-buten-2-ol (*e.g.*, a 2-methyl-3-buten-2-ol synthase). In some aspects, a polypeptide of an isoprene biosynthetic pathway is a 2-methyl-3-buten-2-ol dehydratase polypeptide (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide from *Aquincola tertiaricarbonis*) or variant thereof. In some aspects, the 2-methyl-3-buten-2-ol dehydratase polypeptide is a linalool dehydratase-isomerase polypeptide (*e.g.*, a linalool dehydratase-isomerase polypeptide from *Castellaniella defragrans* Genbank accession number FR669447) or variant thereof. In some aspects, a polypeptide of an isoprene biosynthetic pathway is a 2-methyl-3-buten-2-ol isomerase polypeptide or variant thereof. In some aspects, the 2-methyl-3-butene-2-ol isomerase polypeptide is a linalool dehydratase-isomerase polypeptide (*e.g.*, a linalool dehydratase-isomerase polypeptide from *Castellaniella defragrans* Genbank accession number FR669447) or variant thereof.

[0313] Standard methods can be used to determine whether a polypeptide has the desired isoprene biosynthetic pathway enzymatic activity (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase activity, 2-methyl-3-butene-2-ol isomerase activity, and 3-methyl-2-buten-1-ol activity) by measuring the ability of the polypeptide to convert DMAPP into isoprene *in vitro*, in a cell

extract, or *in vivo*. *See* for example, U.S. Patent Application Publication No.: US 20130309742 A1 and U.S. Patent Application Publication No.: US 20130309741 A1.

[0314] In some aspects, the polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide) is a variant. In some aspects, polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide) is a variant of a wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway. In some aspects, the variant has improved activity such as improved catalytic activity compared to the wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway. 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 polypeptide(s) of an isoprene biosynthetic pathway. 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 polypeptide(s) of an isoprene biosynthetic pathway is a variant of naturally occurring polypeptide(s) of an isoprene biosynthetic pathway and has improved stability (such as thermo-stability) compared to the naturally occurring polypeptide(s) of an isoprene biosynthetic pathway.

[0315] 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 polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide). The variant can share sequence similarity with a wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway. In some aspects, a variant of a wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway 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 polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide). In some aspects, a variant of a wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway 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 polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide).

[0316] In some aspects, the variant comprises a mutation in the wild-type or naturally occurring polypeptide(s) of an isoprene biosynthetic pathway (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide). 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 polypeptide(s) of an isoprene biosynthetic pathway can be one or more, *e.g.* 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more amino acid residues. In some aspects, the nucleic acid encoding the variant (*e.g.*, a 2-methyl-3-buten-2-ol dehydratase polypeptide, 2-methyl-3-butene-2-ol isomerase polypeptide, and 3-methyl-2-buten-1-ol synthase polypeptide) is codon optimized (for example, codon optimized based on host cells where the heterologous polypeptide(s) of an isoprene biosynthetic pathway is expressed).

[0317] 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 polypeptides of an isoprene biosynthetic pathway described herein.

Nucleic acids encoding DXP pathway polypeptides

[0318] 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 through the phosphoketolase 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.

[0319] 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.

[0320] 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

[0321] 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.

[0322] 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*.

[0323] 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*.

[0324] 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*.

[0325] 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*.

[0326] 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*.

[0327] 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*.

[0328] 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

[0329] 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.

[0330] 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.

[0331] In some aspects, the source organism is a yeast, such as *Saccharomyces sp.*, *Schizosaccharomyces sp.*, *Pichia sp.*, or *Candida sp.*

[0332] 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.*

[0333] 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*.

[0334] 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.*

[0335] 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.*

[0336] In some aspects, the source organism is an algae, such as a green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, or dinoflagellates.

[0337] 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

[0338] The recombinant cells described herein (including host cells that have been engineered for increased carbon flux through the phosphoketolase pathway 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In other embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or*

Neosartorya fischeri. In other embodiments, the phosphoketolase polypeptide is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In other embodiments, the phosphoketolase polypeptide is from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cell is a *Corynebacteria spp. (e.g., C. glutamicum).*

[0339] In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus buchneri.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium gallicum.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium dentium.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium bifidum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated

from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.*

[0340] 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.

[0341] In one aspect of the invention, provided herein are recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide as described herein, 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. 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.

[0342] 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 through the phosphoketolase pathway for E4P, GAP, Ac-P, and/or, acetyl-CoA production.

[0343] 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 through the phosphoketolase pathway 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 through the phosphoketolase pathway to mevalonate production.

[0344] 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 through the phosphoketolase pathway 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 through the phosphoketolase pathway to mevalonate production.

[0345] Also provided herein are isoprene-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In other embodiments, the Performance Index value parameters further include (e) isoprene yield protein solubility or (f) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at

least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0346] Additionally provided herein isoprene-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells

comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In other embodiments, the Performance Index value parameters further include (d) isoprene yield protein solubility or (e) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about

0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0347] Further provided herein are isoprene-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In other embodiments, the Performance Index value parameters further include (e) isoprene yield protein solubility or (f) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some

embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0348] Provided herein are isoprene-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In other embodiments, the Performance Index value parameters further include (d) isoprene yield protein solubility or (e) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6,

greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

Methods of using the recombinant cells to produce isoprene

[0349] 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 any phosphoketolase polypeptide as described herein, one or more MVA pathway polypeptides, and an isoprene synthase polypeptide. In certain embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In other

embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.*

[0350] 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 recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides,*

Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*. 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 and/or five carbon sugars such as xylose.

[0351] 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 recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicumi*. In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from

Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, and/or *Mycoplasma arthritidis*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*.

[0352] 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 xylose) 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis,*

Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti, and/or *Mycoplasma columbinum.*

[0353] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*, 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.

[0354] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus,*

Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti, and/or *Mycoplasma columbinum,* 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.

[0355] The recombinant cells described herein that have various enzymatic pathways manipulated for increased carbon flow through the phosphoketolase pathway to mevalonate production can be used to produce isoprene. In some aspects, 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 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*).

[0356] 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.

[0357] 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.

[0358] 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.

[0359] 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}.

[0360] 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 %.

[0361] 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.

[0362] Also provided herein are methods for 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In some embodiments, the phosphoketolase polypeptide is from

Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In yet other embodiments, the phosphoketolase is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In yet other embodiments, the phosphoketolase is from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* 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 through the phosphoketolase pathway 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 through the phosphoketolase pathway to mevalonate production.

[0363] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In some embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri*. In yet other embodiments, the phosphoketolase is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In yet other embodiments, the phosphoketolase is from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*. 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.

[0364] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In some embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri*. In yet other

embodiments, the phosphoketolase is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In yet other embodiments, the phosphoketolase is from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*. In some aspects, the method of producing isoprene further comprises a step of recovering the isoprene.

[0365] Also provided herein are methods for producing isoprene comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said isoprene. In other embodiments, the Performance Index value parameters further include (e) isoprene yield protein solubility or (f) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other

embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0366] Additionally provided herein are methods for producing isoprene comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids

encoding an isoprene synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing said isoprene. In other embodiments, the Performance Index value parameters further include (d) isoprene yield protein solubility or (e) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to

5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0367] Further provided herein are methods for producing isoprene comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing said isoprene. In other embodiments, the Performance Index value parameters further include (e) isoprene yield protein solubility or (f) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41.

In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum.* 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.

[0368] Provided herein are methods for producing isoprene comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing said isoprene. In other embodiments, the Performance Index value parameters further include (d) isoprene yield protein solubility or (e) isoprene specific productivity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than

5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

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

[0369] 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.

[0370] 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.

[0371] 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).

[0372] 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 increase 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

[0373] 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-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

[0374] 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.

[0375] In other embodiments the isoprenoid can be a form of Vitamin A, such as, without limitation, retinol, retinyl palmitate, retinoic acid, alpha-carotene, beta-carotene, gamma-carotene, or the xanthophyll beta-cryptoxanthin. In yet other embodiments, the isoprenoid can be a form of Vitamin E, such as, without limitation a tocopherol (e.g., alpha-tocopherol, beta-

tocopherol, gamma-tocopherol, or delta-tocopherol) or a tocotrienol (e.g., alpha- tocotrienol, beta- tocotrienol, gamma- tocotrienol, or delta- tocotrienol).

Heterologous nucleic acids encoding polyprenyl pyrophosphate synthases polypeptides

[0376] 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 phosphoketolase polypeptide, 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.

[0377] 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.

[0378] 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.

[0379] 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 diphospposphate (GPP) synthase, farnesyl pyrophosphate (FPP) synthase, and geranylgeranyl pyrophosphate (GGPP) synthase, or any other known polyprenyl pyrophosphate synthase polypeptide.

[0380] 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.

[0381] 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.

[0382] 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.

[0383] 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

[0384] The recombinant cells (e.g., recombinant bacterial cells) described herein (including host cells that have been engineered for increased carbon flux through the phosphoketolase pathway as described herein) have the ability to produce isoprenoid precursors and/or isoprenoids at an 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 phosphoketolase polypeptide is from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum*. In other embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides,*

Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In other embodiments, the phosphoketolase polypeptide is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus,* and/or *Mycoplasma arthritidis.* In other embodiments, the phosphoketolase polypeptide is from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti,* and/or *Mycoplasma columbinum.*

[0385] In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus buchneri.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium gallicum.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium dentium.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium bifidum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated

from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In another embodiment, the recombinant cell is a *Corynebacteria spp. (e.g., C. glutamicum).*

[0386] 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 a polyprenyl pyrophosphate 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 isoprenoid precursors and/or isoprenoids compared to isoprenoid precursor and/or isoprenoid -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 isoprenoid precursors and/or isoprenoids, inclusive, as well as any numerical value in between these numbers.

[0387] In one aspect of the invention, provided herein are recombinant cells comprising one or more heterologous nucleic acids encoding a phosphoketolase polypeptide as described herein, 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 a polyprenyl pyrophosphate synthase polypeptide. The cells can further comprise one or more heterologous nucleic acids encoding an IDI

polypeptide. 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 polyprenyl pyrophosphate 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.

[0388] The production of isoprenoids and/or isoprenoid precursors 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, a polyprenyl pyrophosphate synthase polypeptide, MVA pathway polypeptide(s), and/or a DXP pathway polypeptide(s)). As used herein, "enhanced" isoprenoid precursors and/or isoprenoids production refers to an increased cell productivity index (CPI) for isoprenoid precursors and/or isoprenoids, 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 peptide. In certain embodiments described herein, the host cells have been further engineered increased carbon flux through the phosphoketolase pathway for E4P, GAP, Ac-P, and/or, acetyl-CoA production.

[0389] The production of isoprenoid precursors and/or isoprenoids by the recombinant cells described herein can be enhanced by about 5% to about 1,000,000 folds. In certain aspects, 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 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 through the phosphoketolase pathway to MVA production thereby providing enhanced production of isoprenoid precursors and/or isoprenoids as compared to the production of isoprenoid precursors and/or isoprenoids 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 through the phosphoketolase pathway to mevalonate production.

[0390] In other aspects, the production of isoprenoid precursors and/or isoprenoids 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 isoprenoid precursors and/or isoprenoids 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 through the phosphoketolase pathway to MVA production thereby providing enhanced production of isoprenoid precursors and/or isoprenoids as compared to the production of isoprenoid precursors and/or isoprenoids 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 through the phosphoketolase pathway to mevalonate production.

[0391] 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 phosphoketolase polypeptide is from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium*

bifidum, and/or *Clostridium acetobutylicum*. In other embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri*. In other embodiments, the phosphoketolase polypeptide is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis*. In other embodiments, the phosphoketolase polypeptide is from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*. In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus buchneri*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium gallicum*. In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium dentium*. In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium bifidum*. In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum*. In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius,*

Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In another embodiment, the recombinant cell is a *Corynebacteria spp. (e.g., C. glutamicum).* The one or more heterologous nucleic acids can additionally be on one or more vectors.

[0392] 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 phosphoketolase polypeptide is from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In other embodiments, the phosphoketolase polypeptide is from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis,*

Cyanothece sp., and/or Neosartorya fischeri. In other embodiments, the phosphoketolase polypeptide is from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In other embodiments, the phosphoketolase polypeptide is from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Lactobacillus buchneri.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium gallicum.* In yet another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium dentium.* In another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Bifidobacterium bifidum.* In still another embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Clostridium acetobutylicum.* In other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri.* In yet other embodiments, the recombinant cells described herein comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus*

saccharolyticus, Enterococcus casseliflavus, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In one embodiment, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In another embodiment, the recombinant cell is a *Corynebacteria spp. (e.g., C. glutamicum).* 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.

[0393] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*, 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum* 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.

[0394] 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.

[0395] Also provided herein are isoprenoid and/or isoprenoid precursor-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more

polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to

5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0396] Additionally provided herein are isoprenoid and/or isoprenoid precursor-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater

than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0397] Further provided herein are isoprenoid and/or isoprenoid precursor-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-

phosphate. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3

to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0398] Provided herein are isoprenoid precursor and/or isoprenoid-producing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to

SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

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

[0399] 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.

[0400] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum*, 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.

[0401] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum,* 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.

[0402] 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.

[0403] 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.

[0404] 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.

[0405] 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.

[0406] 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 *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum, Clostridium acetobutylicum, Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., Neosartorya fischeri, Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus, Mycoplasma arthritidis, Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum* 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.

[0407] Also provided herein are methods for producing isoprenoid precursors and/or isoprenoids comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing isoprenoid precursors and/or isoprenoids. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4,

greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum.* 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.

[0408] Additionally provided herein are methods for producing isoprenoid precursors and/or isoprenoids comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing isoprenoid precursors and/or isoprenoids. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the polypeptide comprises at least 90%

sequence identity to SEQ ID NO:26. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0409] Further provided herein are methods for producing isoprenoid precursors and/or isoprenoids comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate and producing isoprenoid precursors and/or isoprenoids. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than

5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75, about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

[0410] Provided herein are methods for producing isoprenoid precursors and/or isoprenoids comprising culturing recombinant cells capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cells comprise: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11, (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, and (iii) one or more nucleic acids encoding a polyprenyl pyrophosphate synthase polypeptide, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity and producing isoprenoid precursors and/or isoprenoids. In some embodiments, the polypeptide comprises at least 90% sequence identity to

SEQ ID NO:32. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45. In some embodiments, the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46. In other embodiments, said Performance Index value for any of said parameters are any of such as greater than 1.1, such as greater than 1.2, greater than 1.4, greater than 1.6, greater than 1.8, greater than 2, greater than 2.2, greater than 2.4, greater than 2.6, greater than 2.8, greater than 3, greater than 3.2, greater than 3.4, greater than 3.6, greater than 3.8, greater than 4, greater than 4.2, greater than 4.4, greater than 4.6, greater than 4.8, greater than 5, greater than 5.2, greater than 5.4, greater than 5.6, greater than 5.8, greater than 6, greater than 6.2, greater than 6.4, greater than 6.6, greater than 6.8, greater than 7, greater than 7.2, greater than 7.4, greater than 7.6, greater than 7.8, greater than 8, greater than 8.2, greater than 8.4, greater than 8.6, greater than 8.8, 9, greater than 9.2, greater than 9.4, greater than 9.6, greater than 9.8, or greater than 10 or more compared to a parental polypeptide having phosphoketolase activity (*e.g.,* a phosphoketolase from *E. gallinarum*). In other embodiments, cell performance index increases 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 comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 0.5, about 0.25 to 0.75, about 0.5 to 1, about 0.75 to 1.25, about 1 to 1.5, about 1.25 to 1.75, about 1.5 to 2, about 1.75 to 2.25, about 2 to 2.5, about 2.25 to 2.75, about 2.5 to 3, about 2.75 to 3.25, about 3 to 3.5, about 3.25 to 3.75, about 3.5 to 4, about 3.75 to 4.25, about 4 to 4.5, about 4.25 to 4.75,

about 4.5 to 5, about 4.75 to 5.25, about 5 to 5.5, about 5.25 to 5.75, about 5.5 to 6, about 6.25 to 6.75, about 6.5 to 7, about 6.75 to 7.25, about 7 to 7.5, about 7.75 to 8.25, about 8 to 8.5, about 8.25 to 8.75, about 8.5 to 9, about 8.75 to 9.25, about 9 to 9.5, about 9.25 to 9.75, or about 9.5 to 10 or more in comparison to a parental molecule. In other embodiments, the cell performance index is greater than any of about 0.1 to 2, about 1-3, about 2-4, about 3-5, about 4-6, about 5-7, about 6-8, about 7-9, or about 8-10 or more in comparison to a parental molecule. In some embodiments, the parental molecule is a phosphoketolase from *E. gallinarum*. 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.

Vectors

[0411] 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.

[0412] 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

[0413] Nucleic acids encoding 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, 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

[0414] 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.

[0415] Any cell or progeny thereof that can be used to heterologously express genes can be used to express one or more a phosphoketolase. In certain embodiments, the cells (e.g., recombinant cells) comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti,*

Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides, Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus, Mycoplasma alligatoris, Carnobacterium sp., Melissococcus plutonius, Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae, Mycoplasma agalactiae, Streptococcus gordonii, Kingella oralis, Mycoplasma fermentans, Granulicatella adiacens, Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24.

[0416] The cells (e.g., recombinant cells) with heterologous nucleic acid encoding a phosphoketolase as described above and herein can also be engineered 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 *Corynebacteria (e.g. C. glutamicum)*, *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.*

[0417] 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.

[0418] 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.

[0419] 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. Facultative anaerobic 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 alternatively grown in the presence of greater amounts of oxygen.

[0420] 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.

[0421] 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.

[0422] 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.

[0423] *E. coli* host cells can be used to express one or more phosphoketolase enzymes from any number of organisms. In certain embodiments, the cells (e.g., recombinant cells) comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Burkholderia phytofirmans, Lactobacillus buchneri, Bifidobacterium gallicum, Bifidobacterium dentium, Bifidobacterium bifidum*, and/or *Clostridium acetobutylicum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Mycobacterium gilvum, Shewanella baltica, Lactobacillus rhamnosus, Lactobacillus crispatus, Bifidobacterium longum, Leuconostoc citreum, Bradyrhizobium sp., Enterococcus faecium, Brucella microti, Lactobacillus salivarius, Streptococcus agalactiae, Rhodococcus imtechensis, Burkholderia xenovorans, Mycobacterium intracellulare, Nitrosomonas sp., Schizosaccharomyces pombe, Leuconostoc mesenteroides,*

Streptomyces sp., Lactobacillus buchneri, Streptomyces ghanaensis, Cyanothece sp., and/or Neosartorya fischeri. In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Enterococcus faecium, Listeria grayi, Enterococcus gallinarum, Enterococcus saccharolyticus, Enterococcus casseliflavus*, *Mycoplasma alligatoris*, *Carnobacterium sp., Melissococcus plutonius*, *Tetragenococcus halophilus*, and/or *Mycoplasma arthritidis.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from *Streptococcus agalactiae*, *Mycoplasma agalactiae*, *Streptococcus gordonii*, *Kingella oralis, Mycoplasma fermentans*, *Granulicatella adiacens*, *Mycoplasma hominis, Mycoplasma crocodyli, Mycobacterium bovis, Neisseria sp., Streptococcus sp., Eremococcus coleocola, Granulicatella elegans, Streptococcus parasanguinis, Aerococcus urinae, Kingella kingae, Streptococcus australis, Streptococcus criceti*, and/or *Mycoplasma columbinum.* In some embodiments, the recombinant cells comprise one or more copies of a heterologous nucleic acid encoding a phosphoketolase isolated from and organism listed in Table 1, Table 2 and/or Figures 3-24.

[0424] These cells can also be engineered with one or more heterologous nucleic acids encoding one or more MVA pathway polypeptides, isoprene synthase, IDI, DXP pathway polypeptide(s), 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 described above and herein 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 described above and herein 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 described above and herein 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 or more phosphoketolase enzymes are any phosphoketolase enzymes as disclosed herein.

Exemplary Host Cell Modifications

Citrate Synthase Pathway

[0425] 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).

[0426] 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

[0427] 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.

[0428] 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.

[0429] 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 an 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.

[0430] 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.

[0431] 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

[0432] 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.

[0433] 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.

[0434] 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%.

[0435] 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

[0436] 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).

[0437] 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

[0438] 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).

[0439] 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.

[0440] 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.

[0441] 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%.

[0442] 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.

[0443] 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

[0444] *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).

[0445] 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.

[0446] 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 increase 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 decrease 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.

[0447] 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

[0448] 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).

[0449] 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.

[0450] 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

[0451] 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.

[0452] 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.

[0453] 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.

[0454] 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

[0455] 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.

[0456] 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

[0457] In certain embodiments described herein, the utilization of xylose is desirable to convert sugar 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.

[0458] 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 endogenous 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).

[0459] 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 endogenous 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

[0460] 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 endogenous 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

[0461] 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

[0462] 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.

[0463] 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.

[0464] In other aspects, the host cells can be further engineered to increase intracellular acetyl-phosphate 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).

[0465] 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.

[0466] 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.

[0467] 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

[0468] 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.

[0469] 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.

[0470] 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.

[0471] 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).

[0472] 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.

[0473] 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).

Exemplary Cell Culture Conditions

[0474] 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.

[0475] 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.

[0476] 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.

[0477] 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.

[0478] 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

[0479] 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.

[0480] Suitable purification methods are described in more detail in U.S. Patent Application Publication US2010/0196977 A1.

[0481] 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.

[0482] 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: Identification of phosphoketolases

[0483] To identify phosphoketolases that could be used for improved production of acetyl coenzyme A-derived (acetyl-CoA-derived) metabolites, isoprene, isoprenoid precursors, and isoprenoids in recombinant cells, the CDART program within the NCBI website was used to select all gene products that were consistent with the known phosphoketolase domain architecture (Geer L et al. (2002), *"CDART: protein homology by domain architecture."*, **Genome Res.12(10)**1619-23). Sequences were further refined by selecting the refseq sequences from the original domain architecture search. Next, the sequences were clustered into 22 distinct groups based on sequence similarity (Clustering by Passing Messages Between Data Points. Brendan J. Frey and Delbert Dueck, University of Toronto *Science* **315**, 972–976, February 2007). Briefly, the amino acid sequences were multiply aligned using ClustalW. Pairwise percent identities (PIDs) were calculated. This was operationally defined and in this case it was the number of residues that were identical over residues that were aligned. The PIDs were converted to distances by way of the formula K = -Ln(1 - D - (D.D)/5) (Kimura, M. The neutral Theory of Molecular Evolution, Camb.Univ.Press, 1983, page 75). Negative distances were used as similarity score in the above algorithm. Medium similarities were used as preferences for each data point. 22 clusters were defined using this method (Figures 3-24). DNA encoding the amino acid sequence of the central representative sequence from each cluster was synthesized (Figure 2 and Table 1). In cases where the central representative from a cluster was determined to be unlikely to represent an active phosphoketolase due to the absence of complete

phosphoketolase domains, an alternate phosphoketolase from that cluster was selected for DNA synthesis (Table 1).

Table 1. Central representative sequence

Cluster	Organism	NCBI identifier number	Amino acid SEQ ID NO	Nucleic Acid SEQ ID NO
1	Mycobacterium gilvum Spyr1	315444259	1	52
2	Shewanella baltica OS185	152999647	2	53
3	Lactobacillus rhamnosus LMS2-1	229550902	3	54
4	Lactobacillus crispatus ST1	295692465	4	55
5	Bifidobacterium longum subsp. longum JDM301	296453922	5	N/D
6	Leuconostoc citreum KM20	170016535	6	56
7	Bradyrhizobium sp. S23321	383773704	7	57
8	Enterococcus faecium E1039	293556655	8	N/D
9	Brucella microti CCM 4915	256015169	9	58
10	Lactobacillus salivarius ATCC 11741	227891468	10	59
11	Streptococcus agalactiae COH1	77409068	11	N/D
12	Rhodococcus imtechensis RKJ300	384105306	12	60
13	Burkholderia xenovorans LB400	91778759	13	61
14	Mycobacterium intracellulare ATCC 13950	254819329	14	62
15	Nitrosomonas sp. Is79A3	339481558	15	63
16*	Schizosaccharomyces pombe 972h-	19112755	16	64

17	Leuconostoc mesenteroides subsp. mesenteroides J18	381336925	17	N/D
18	Streptomyces sp. SA3_actG	318056880	18	N/D
19	Lactobacillus buchneri ATCC 11577	227512366	19	65
20	Streptomyces ghanaensis ATCC 14672	291440956	20	66
21	Cyanothece sp. PCC 8802	257059544	21	67
22	Neosartorya fischeri NRRL 181	119473535	22	68

N/D indicates not done
* Replaced the central representative Aspergillus fumigatus Af293 (NCBI number 70999652)

[0484] DNA encoding the protein sequences that were less than 90% identical to each other by pairwise alignment using ClustalW within Cluster 8, which contained the *Enterococcus gallinarum* phosphoketolase, and to Cluster 11, which shared the most homology with Cluster 8, were designed for protein synthesis (Table 2).

Table 2. Sequences from Cluster 8 and Cluster 11

Organism	NCBI identifier number	Amino acid SEQ ID NO	Nucleic Acid SEQ ID NO
Cluster 8			
Enterococcus faecium TX1330	227551751	23	69
Listeria grayi DSM 20601	299821157	24	70
Enterococcus casseliflavus EC30	257866707	25	71
Mycoplasma alligatoris A21JP2	293363787	26	72
Carnobacterium sp. 17-4	328958427	27	73
Melissococcus plutonius ATCC 35311	332686509	28	74
Tetragenococcus halophilus NBRC 12172	352516443	29	75

Melissococcus plutonius DAT561	379727960	30	76
Mycoplasma arthritidis 158L3-1	193216764	31	77
Cluster 11			
Streptococcus agalactiae NEM316	25011879	32	78
Mycoplasma agalactiae PG2	148377390	33	79
Streptococcus gordonii str. Challis substr. CH1	157150221	34	80
Kingella oralis ATCC 51147	238021480	35	81
Mycoplasma fermentans M64	319776755	36	82
Granulicatella adiacens ATCC 49175	259046526	37	83
Mycoplasma hominis ATCC 23114	269115076	38	84
Mycoplasma crocodyli MP145	294155803	39	85
Neisseria sp. oral taxon 014 str. F0314	298369811	40	86
Eremococcus coleocola ACS-139-V-Col8	313884493	41	87
Aerococcus urinae ACS-120-V-Col10a	326803378	42	88
Kingella kingae ATCC 23330	333376439	43	89
Streptococcus criceti HS-6	357236206	44	90
Streptococcus criceti HS-6	357235889	45	91
Mycoplasma columbinum SF7	343491865	46	92

Table 3. Sequences from Cluster 8—Amino Acid Percent Sequence Identity

Cluster 8 Reference AA	**Phosphoketolase AA Sequence**	**Amino Acid % Identity**
SEQ ID NO:8	SEQ ID NO:23	98
SEQ ID NO:8	SEQ ID NO:24	73
SEQ ID NO:8	SEQ ID NO:25	74
SEQ ID NO:8	SEQ ID NO:26	67
SEQ ID NO:8	SEQ ID NO:27	71
SEQ ID NO:8	SEQ ID NO:28	72
SEQ ID NO:8	SEQ ID NO:29	70
SEQ ID NO:8	SEQ ID NO:30	72
SEQ ID NO:8	SEQ ID NO:31	70

Table 4: Sequences from Cluster 11—Amino Acid Percent Sequence Identity

Cluster 11 Reference AA	Phosphoketolase AA Sequence	Amino Acid % Identity
SEQ ID NO:11	SEQ ID NO:32	99
SEQ ID NO:11	SEQ ID NO:33	65
SEQ ID NO:11	SEQ ID NO:34	89
SEQ ID NO:11	SEQ ID NO:35	74
SEQ ID NO:11	SEQ ID NO:36	69
SEQ ID NO:11	SEQ ID NO:37	79
SEQ ID NO:11	SEQ ID NO:38	65

SEQ ID NO:11	SEQ ID NO:39	68
SEQ ID NO:11	SEQ ID NO:40	77
SEQ ID NO:11	SEQ ID NO:41	67
SEQ ID NO:11	SEQ ID NO:42	68
SEQ ID NO:11	SEQ ID NO:43	74
SEQ ID NO:11	SEQ ID NO:44	84
SEQ ID NO:11	SEQ ID NO:45	79
SEQ ID NO:11	SEQ ID NO:46	66

Example 2: Identification of phosphoketolases in bacterial genomes lacking phosphofructokinase

[0485] A search was conducted for bacterial genomes that had an annotated phosphoketolase (PKL) but did not have an annotated phosphofructokinase (PFK), a critical enzyme for carbon flux through glycolysis. Several organisms that fit these criteria, and from this list five PKLs, specifically PKLs from *Burkholderia phytofirmans* PsJN (SEQ ID NO:47), *Lactobacillus buchneri* NRRL B-30929 (SEQ ID NO:48), *Bifidobacterium gallicum* DSM 20093 (SEQ ID NO:49), *Bifidobacterium dentium* Bd1 (SEQ ID NO:50), and *Bifidobacterium bifidum* IPLA 20015 (SEQ ID NO:51), were chosen for investigation of high activity and increased yield of isoprene from glucose. Since most of the PKLs from the full list of organisms have not been characterized, the five PKLs that were chosen were based on sequence diversity and the best circumstantial evidence of high activity that could be obtained in the literature. The PKL from *Bifidobacterium dentium* displayed a pH optimum of 7 (Sgorbati B., et al., *Antonie van Leeuwenhoek* **1976** (42), 49-57), whereas the pH optima for other PKLs is typically around 6 (Heath EC., et al., *J Bio Chem* **1957**, 1009-1029). *Lactobacillus buchneri* was isolated as a contaminant from a fuel ethanol plant, and was shown to grow on both glucose and xylose, presumably by activity of PKL on either F6P or X5P for cell mass and energy (Liu S., et al., *J Ind Microbiol Biotechnol* **2008** (35), 75-81). The PKLs from *Bifidobacterium bifidum* and *Bifidobacterium gallicum* were chosen because these strains were able to grow well on either glucose or xylose as the sole carbon source (Palframan RJ., et al., *Curr Issues Intest Microbiol* **2003** (4), 71-75).

Example 3: Cloning of identified phosphoketolase enzymes

[0486] PKLs obtained from *Bifidobacterium longum* subsp. *infantis, Enterococcus gallinarum*, and *Clostridium acetobutylicum* were each assayed for enzyme activity. *Bifidobacterium longum* subsp. *infantis* PKL had a Km of 5.7 ± 1.16 mM, a kcat of 4.56 ± 0.2 sec^{-1}, and a kcat/Km of 0.79 ± 0.2 mM^{-1} sec^{-1}, *Enterococcus gallinarum* PKL had a Km of 10.4 ± 1.03 mM, a kcat of 1.35 ± 0.04 sec^{-1}, and a kcat/Km of 0.13 ± 0.1 mM^{-1} sec^{-1}, and *Clostridium acetobutylicum* PKL was found to have a Km of 10.3 ± 0.67 mM, a kcat of 2.18 ± 0.05 sec^{-1}, and a kcat/Km of 0.21 ± 0.06 mM^{-1} sec^{-1}. A construct encoding the *Bifidobacterium longum* subsp. *infantis, Enterococcus gallinarum*, or *Clostridium acetobutylicum* PKLs was used as a control to screen the candidate PKL enzymes for *in vitro* and *in vivo* activity.

[0487] The amino acid sequence of *Enterococcus gallinarum* PKL (SEQ ID NO: 93) was obtained from GenBank 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 BspHI 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 was then subcloned into a NcoI/SacI-digested pTrcHis2B vector (Life Technologies, Carlsbad, CA) to form plasmid pCMP1321 (Table 5, Figure 25).

[0488] Chromosomal DNA of strain ATCC15697, *Bifidobacterium longum* subsp. *infantis* was obtained from ATCC (Manassas, VA). The gene encoding *B. longum* PKL was amplified by polymerase chain reaction (PCR) from the chromosomal DNA using primers CMP283: 5'-ctgtatTCATGAcgagtcctgttattggcacc-3' and CMP284: 5'-ctctatGAATTCTCACTCGTTGTCGCCAGCG-3', 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 approximately 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 (Table 5).

[0489] For construction of the control plasmid encoding a *Clostridium acetobutylicum* PKL, chromosomal DNA of strain ATCC BAA-98 was obtained from ATCC (Manassas, VA). The gene encoding *Clostridium acetobutylicum* PKL was amplified by polymerase chain reaction

(PCR) from the chromosomal DNA using primers CacetpTrcHisBF: 5'-taaggaggaataaaccatgcaaagtataataggaaaacataaggatgaagg-3' and CacetpTrcHisBR: 5'-ttctagaaagcttcgttatacatgccactgccaattagttatttc-3', and the polymerase Herculase according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). The PCR product was purified and assembled into EcoRI/NcoI-digested pTrcHis2B (Invitrogen, Carlsbad, CA) using the GENEART seamless cloning kit (Invitrogen, Carlsbad, CA) to form plasmid pCMP1364 (Table 3).

[0490] The nucleic acid sequence encoding a PKL protein derived from each of *Bifidobacterium dentium*, *Bifidobacterium bifidum*, *Bifidobacterium gallicum*, *Lactobacillus buchneri*, *Burkholderia phytofermans*, and *Clostridium acetobutylicum* (SEQ ID NO:94) were codon optimized for expression in E. coli, and synthesized by Gene Oracle (Mountain View, CA). These codon-optimized PKL genes were amplified by PCR and subcloned into the pTrcHis2B expression plasmid using the GeneArt Seamless Cloning Kit (Life Technologies), according to the manufacturer's recommended protocol. Table 5 below lists the primers used for construction of plasmids pMCS530 through pMCS535. The PKL enzymes were cloned downstream of the pTrc promoter to permit inducible expression of the phosphoketolase genes by IPTG (Table 6, Figures 26-31).

Table 5. Primers used for construction of plasmids

Primer	Sequence	Description
Bifidobacterium dentium		
o430	tgataacgaataagagctcgagatctgcagctggtacc	DentiumPKL into pTrcHis2B, plasmid Forward primer
o431	gactcgtcatggtttattcctccttatttaatcgatacattaatatatacc	DentiumPKL into pTrcHis2B, plasmid Reverse primer
o432	ggaataaaccatgacgagtccagttattggaacaccc	DentiumPKL into pTrcHis2B, PKL Forward primer
o433	tctcgagctcttattcgttatcacccgcagtagcgg	DentiumPKL into pTrcHis2B, PKL Reverse primer
Bifidobacterium bifidum		
o434	cgacaacgagtaagagctcgagatctgcagctggtacc	Bifidum PKL into pTrcHis2B, plasmid Forward primer
o435	gagaggtcatggtttattcctccttatttaatcgatacattaatatatacc	Bifidum PKL into pTrcHis2B, plasmid Reverse primer
o436	ggaataaaccatgacctctccagtaattggcactcc	Bifidum PKL into pTrcHis2B, PKL Forward primer
o437	tctcgagctcttactcgttgtcgcctgccgtg	Bifidum PKL into pTrcHis2B, PKL Reverse primer

Bifidobacterium gallicum		
o438	cgataatgaataagagctcgagatctgcagctggtacc	Gallicum PKL into pTrcHis2B, plasmid Forward primer
o439	gagaagtcatggtttattcctccttatttaatcgatacattaatatatacc	Gallicum PKL into pTrcHis2B, plasmid Reverse primer
o440	ggaataaaccatgacttctcccgtgattggtactcc	Gallicum PKL into pTrcHis2B, PKL Forward primer
o441	tctcgagctcttattcattatcgcccgccgtagc	Gallicum PKL into pTrcHis2B, PKL Reverse primer
Lactobacillus buchneri		
o442	gctgaaaaaataagagctcgagatctgcagctggtacc	Buchneri PKL into pTrcHis2B, plasmid Forward primer
o443	ccactgtcatggtttattcctccttatttaatcgatacattaatatatacc	Buchneri PKL into pTrcHis2B, plasmid Reverse primer
o444	ggaataaaccatgacagtggactatgactcaaaagagtacttagag	Buchneri PKL into pTrcHis2B, PKL Forward primer
o445	tctcgagctcttatttttcagcccttcccatttcc	Buchneri PKL into pTrcHis2B, PKL Reverse primer
Burkholderia phytofermans		
o446	ctggaaaggttaagagctcgagatctgcagctggtacc	Phytofermans PKL into pTrcHis2B, plasmid Forward primer
o447	cttcagccatggtttattcctccttatttaatcgatacattaatatatacc	Phytofermans PKL into pTrcHis2B, plasmid Reverse primer
o448	ggaataaaccatggctgaagccactgcccatc	Phytofermans PKL into pTrcHis2B, PKL Forward primer
o449	tctcgagctcttaacctttccaggtccaattccggattt	Phytofermans PKL into pTrcHis2B, PKL Reverse primer
Clostridium acetobutylicum		
o450	atggcatgtataagagctcgagatctgcagctggtacc	Acetobutylicum optimized PKL into pTrcHis2B, plasmid Forward primer
o451	ttgattgcatggtttattcctccttatttaatcgatacattaatatatacc	Acetobutylicum optimized PKL into pTrcHis2B, plasmid Reverse primer
o452	ggaataaaccatgcaatcaatcatcggcaaacac	Acetobutylicum optimized PKL into pTrcHis2B, PKL Forward primer
o453	tctcgagctcttatacatgccattgccagtttgtgatc	Acetobutylicum optimized PKL into pTrcHis2B, PKL Reverse primer

Table 6. Plasmids encoding PKLs

Plasmid	Description
pCMP1321	pTrcHis2B *E. gallinarum* PKL, Carb
pCMP1090	pTrcHis2B *B. longum* PKL, Carb
pCMP1364	pTrcHis2B *C. acetobutylicum* PKL, Carb
pMCS530	pTrcHis2B *B. dentium* PKL, Carb

pMCS531	pTrcHis2B *B. bifidum* PKL, Carb
pMCS532	pTrcHis2B *B. gallicum* PKL, Carb
pMCS533	pTrcHis2B *L. buchneri* PKL, Carb
pMCS534	pTrcHis2B *B. phytofermans* PKL, Carb
pMCS535	pTrcHis2B *C. acetobutylicum* PKL optimized, Carb

Carb indicates carbenicillin

[0491] The nucleic acid sequence encoding a PKL protein derived from each of the organisms listed in Table 1, Table 2, and Clusters 1-22 (see Figures 3-24) are codon optimized for expression in *E. coli* and synthesized. These codon-optimized PKL genes are subcloned into the pTrcHis2B expression plasmid downstream of the pTrc promoter to permit inducible expression of the phosphoketolase gene by IPTG.

Example 4: Construction of strains expressing identified PKLs for *in vitro* studies

[0492] PKL expressing strains were constructed by transforming strain CMP1133 (BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA) with the plasmids listed on Table 5 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 7).

Table 7: Description of *E. coli* strains

Strain Name	Genotype
CMP1183	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1090 (pTrcPKL *B. longum*)
CMP1328	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1321 (pTrcPKL *E. gallinarum*)
CMP1366	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pCMP1364 (pTrcPKL *C. acetobutylicum*)
MCS545	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pMCS530 (pTrcPKL *B. dentium*)
MCS546	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS,

	thiFRTtruncIspA, pMCS531 (pTrcPKL *B. bifidum*)
MCS547	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pMCS532 (pTrcPKL *B. gallicum*)
MCS548	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pMCS533 (pTrcPKL *L. buchneri*)
MCS549	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pMCS534 (pTrcPKL *B. phytofermans*)
MCS550	BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA, pMCS535 (pTrcPKL *C. acetobutylicum optimized*)

[0493] PKL expressing strains, each expressing an identified PKL, are constructed by transforming strain CMP1133 (BL21, Δpgl PL.2mKKDyl, GI1.2gltA, yhfSFRTPyddVIspAyhfS, thiFRTtruncIspA) with a plasmid encoding a PKL listed on Table 1, Table 2, and Clusters 1-22 (see Figures 3-24) and is selected for colonies on Luria-Bertani plates containing 20 μg/ml kanamycin. The kanamycin marker is removed using the protocol recommended by the manufacturer (Gene Bridges, Heidelberg, Germany).

Example 5: Comparison of expression and solubility of identified PKLs

[0494] Strains expressing pTrcHis2B *B. longum* (strain CMP1183), pTrcHis2B *E. gallinarum* (strain CMP1328), pTrcHis2B *C. acetobutylicum* (strain CMP1366), pTrcHis2B *B. dentium* PKL (strain MCS545), pTrcHis2B *B. bifidum* (strain MCS546), pTrcHis2B *B. gallicum* PKL (strain MCS547), pTrcHis2B *L. buchneri* PKL (strain MCS548), pTrcHis2B *B. phytofermans* PKL (strain MCS549), or pTrcHis2B *C. acetobutylicum* PKL optimized (strain MCS550) 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. Supernatant and pellet samples were analyzed by 4-12% SDS-PAGE gel electrophoresis. Solubility was assessed by comparison of soluble versus pellet (insoluble) phosphoketolase fractions.

[0495] The results showed that optimized *C. acetobutylicum* PKL (Fig. 32A, lane 9) was expressed at a higher level as compared to *C. acetobutylicum* PKL that had not been codon-optimized (Fig. 32A, lane 3). *B. dentium* (Fig. 32A, lane 4), *B. bifidium* (Fig. 32A, lane 5), and *B. gallicum* (Fig. 32A, lane 6) PKLs were all expressed at a similar level to *C. acetobutylicum* PKL (Fig. 32A, lane 3) and were mostly soluble (Fig. 32B). In comparison, *L. buchneri* (lane 7) and *B. phytofermans* (lane 8) were almost completely insoluble (Fig. 32B).

[0496] Strains expressing an identified PKL listed on Table 1, Table 2, and Clusters 1-22 (see Figures 3-24) are 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 are harvested by centrifuging 4 ml culture broth at 3000 rpm for 10 minutes. Cell pellets are re-suspended in 2ml of 50mM MES, 50mM NaCl pH6.0 with 0.1% DNAase and 0.5mM AEBSF. The cell suspension is lysed using a french pressure cell at 14,000 psi (American Instrument Company). The lysate is then centrifuged at 15,000 RPM for 10 minutes at 4°C in an Eppendorf 5804R centrifuge. The supernatant and pellet are separated. The pellets are resuspended in the lysis buffer (50mM MES, 50mM NaCl pH6.0). Supernatant and pellet samples are analyzed by 4-12% SDS-PAGE gel electrophoresis. Solubility is assessed by comparison of soluble versus pellet (insoluble) phosphoketolase fractions.

Example 6: *In vitro* screen for phosphoketolase activity in strains expressing identified PKLs

[0497] Strains expressing pTrcHis2B *B. longum* (strain CMP1183), pTrcHis2B *E. gallinarum* (strain CMP1328), pTrcHis2B *C. acetobutylicum* (strain CMP1366), pTrcHis2B *B. dentium* PKL (strain MCS545), pTrcHis2B *B. bifidum* (strain MCS546), pTrcHis2B *B. gallicum* PKL (strain MCS547), pTrcHis2B *L. buchneri* PKL (strain MCS548), pTrcHis2B *B. phytofermans* PKL (strain MCS549), or pTrcHis2B *C. acetobutylicum* PKL optimized (strain MCS550) were grown in LB medium with 50 μg/ml carbenicillin at 37°C prior to induction. Following induction with 10 μM, 25 μM, 50 μM, or 100 μM IPTG, cultures were transferred to a 34°C shaker for 30 minutes. Cells were harvested by centrifugation at 10,000 rpm for 10 min at 4°C. Cell pellets were stored at -80°C prior to purification. For purification, 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 the PKL from *B. longum*, *E. gallinarum*, *C. acetobutylicum*, *B. dentium*, *B. bifidum*, *B. gallicum*, *L. buchneri*, *B. phytofermans*, or *C. acetobutylicum* were 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. The amount of AcP formed by each PKL was 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 their 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, and absorbance changes associated with the amount of AcP formed were monitored at 505 nm.

[0498] The results showed that optimized *C. acetobutylicum* PKL had F6P activity and produced greater amounts of AcP as compared to *C. acetobutylicum* PKL that had not been codon-optimized (Fig. 33). *B. dentium* had similar PKL F6P activity as *C. acetobutylicum* PKL that had not been codon-optimized. *B. dentium* and *B. gallicum* PKLs had significant F6P activity and were comparable to *E. gallinarum* PKL F6P activity. In comparison, *L. buchneri* PKL (Fig. 33) and *B. phytofermans* PKL did not demonstrate F6P activity which is supported by the finding that these PKLs are almost completely insoluble.

[0499] Strains expressing an identified PKL listed on Table 1, Table 2, and Clusters 1-22 (see Figures 3-24) are grown in LB medium with 50 µg/ml carbenicillin at 37°C prior to induction. Following induction with 10 µM, 25 µM, 50 µM, or 100 µM IPTG, cultures are transferred to a 34°C shaker for 30 minutes. Cells are harvested by centrifugation at 10,000 rpm for 10 min at

4°C. For purification, PKL cell pellets are resuspended in 50 mM MES pH 6.0, 50 mM NaCL, 0.5 mM AEBSF, 0.1 mg/ml DNaseI. Cells are lysed by repeated passage through a French press and clarified by ultracentrifugation at 50,000rpm for 60 min. Clarified lysate containing the PKLs are 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 are analyzed by SDS-PAGE. Fractions containing PKL are pooled and desalted using a G25 desalting column into 50 mM MES, 50 mM NaCL pH 6.0. Further purification is 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. The amount of AcP formed by each PKL is 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. The assays are performed in a 96-well plate (Costar catalog #9017) format, at 37°C. Each 300 µl reaction contains 1mM TPP, 10 mM potassium phosphate pH 6.0, 50 mM MES pH 6, 10 mM MgCl2, 5 mM F6P and PKL at a concentration of 250 nM. Time points are taken at various intervals. In order to stop the reaction, 60 µl of the reaction mixture is 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 is used to precipitate the protein and allow AcP detection. The samples are then centrifuged at 3000 rpm for 10 min. A 200 µl sample of supernatant is transferred to a microtiter plate and a plate reader, and absorbance changes associated with the amount of AcP formed are monitored at 505 nm.

Example 7: *In vivo* screen for phosphoketolase activity in strains expressing identified Phosphoketolases (PKLs)

[0500] The *in vivo* activities of phosphoketolase (PKL) enzymes were evaluated in a mutant strain that has no transketolase (*tkt*) activity. Transketolase is responsible for producing erythrose-4-phosphate (E4P), the substrate for all aromatic vitamins and amino acids in *E. coli*. Growth of *E. coli* on minimal medium with glucose as a carbon source in the absence of transketolase activity is therefore not possible due to aromatic auxotrophy (Zhao and Winkler 1994). Transketolase is also involved in the interconversion of xylulose-5-phosphate (X5P) with sedoheptulose-7-phosphate (S7P) and glyceraldehyde-3-phosphate (GAP), and growth of a *tkt* mutant on minimal medium with xylose as a carbon source is also not possible, since *tkt* activity is the only outlet back into glycolysis from the pentose phosphate pathway. Since

phosphoketolase produces E4P from F6P, and GAP from X5P, functional enzymes can rescue the growth defects of a *tkt* mutant when grown on glucose (indicating F6P activity) or xylose (indicating both X5P and F6P activity). Growth of complemented mutants therefore can be used to test the different *in vivo* activities of phosphoketolase enzymes.

Strain Construction

[0501] Standard molecular biology techniques to amplify mutations from the Keio collection by PCR, perform P1 transduction, perform GeneBridges insertions (manufacturer's protocol), PCR amplification (Pfu Turbo or Herculase, manufacturer's protocol), transform plasmids, and to grow and propagate strains were used. Briefly, since there are two transketolase enzymes in the genome of *E. coli*, both had to be knocked out to generate a transketolase null mutant. The kanamycin insert in *tktB* was amplified by PCR from the Keio collection and introduced by recombineering into BL21. The antibiotic resistance cassette in *tktB* was confirmed by PCR and then looped out using the pCP20 plasmid (Table 5). The *tktA* mutation was then introduced into BL21 by the same method and subsequently introduced into the *tktB* mutant by P1 transduction to generate a transketolase null mutant strain (Table 6). This strain, DW809, only grew on M9 glucose minimal medium with casamino acids that did not contribute substantially to the aromatic amino acid supply and an additional supplement of all aromatic compounds, including tyrosine, phenylalanine, tryptophan, p-aminobenzoate, 2-3-dihydroxybenzoate, p-hydroxybenzoate, and pyridoxine (as indicated in Zhao and Winkler, 1994). This combination of six aromatic compounds and pyridoxine is subsequently referred to herein as the "aromatic supplement." Plasmids harboring different phosphoketolase enzymes were then transformed into the transketolase mutant strain, and selected for growth on M9 glucose casamino acids with the aromatic supplement and carbenicillin (Table 6). Strains were then assayed for growth on an Enzyscreen Growth Profiler (Enzyscreen, BV) on either M9 glucose or xylose without the aromatic supplement and compared to the control strain that did not express a phosphoketolase enzyme. Phosphoketolase enzymes were induced in the transketolase null mutant at two different concentrations of IPTG, 20 μM and 60 μM.

[0502] The transektolase null mutant strain is transformed with an identified PKL listed on Table 1, Table 2, or Clusters 1-22 (see Figures 3-24) and selected for growth on M9 glucose casamino acids with the aromatic supplement and carbenicillin. Strains are then assayed for growth on an Enzyscreen Growth Profiler (Enzyscreen, BV) on either M9 glucose or xylose

without the aromatic supplement and compared to the control strain that did not express a phosphoketolase enzyme. Phosphoketolase enzymes are induced in the transketolase null mutant at two different concentrations of IPTG, 20 μM and 60 μM.

Table 8. Primers for testing presence of *tktA* and *tktB* mutations

Primer Name	**Sequence**
tktA test for	catgcgagcatgatccagagatttctga
tktA test rev	gcttgtccgcaaacggacatatcaaggt
tktB test for	cagctcccatgagcgaagcggagt
tktB test rev	gacgcgtcagcgtcgcatccggca
tktB B test for	gctgcgatcgactgactatcgcaccga
tktB B test rev	cagacgcctggcccacgttgtggatca
tktA B test for	gcagcggacgggcgagtagattgcgca
tktA B test rev	gtgatctacaacacgccttatctat

Table 9. Engineered strains expressing PKLs

Strain	Description
DW809	BL21 ΔtktA ΔtktB, Kan (antibiotic marker in tktA from Keio)
DW816	BL21 ΔtktA ΔtktB, pCMP1321, Kan Carb
DW830	BL21 ΔtktA ΔtktB, pMCS530, Kan Carb
DW831	BL21 ΔtktA ΔtktB, pMCS531, Kan Carb
DW832	BL21 ΔtktA ΔtktB, pMCS532, Kan Carb
DW833	BL21 ΔtktA ΔtktB, pMCS533, Kan Carb
DW834	BL21 ΔtktA ΔtktB, pMCS534, Kan Carb
DW835	BL21 ΔtktA ΔtktB, pMCS535, Kan Carb

Results

[0503] In this assay, the transketolase mutant grew on glucose only with supplement (Figure 34) and did not grown on xylose with or without supplement (Figure 36). Growth of the transketolase null mutant expressing different phosphoketolases highlighted the differential *in vivo* behavior of these enzymes. *E. gallinarum* PKL displayed the best performance on both glucose and xylose, indicating sufficient F6P and X5P activity to maintain growth of the transketolase mutant in the absence of supplement (see Figures 35 and 37). The *C. acetobutylicum* PKL also allowed for growth of the transketolase mutant in the absence of aromatic supplement on glucose and xylose (Figure 35 and 37), but appeared to have a deleterious effect on cell growth at the 60 μM IPTG concentration when grown on glucose (Figure 35).

Example 8: Measurement of intracellular acetyl phosphate in strains expressing PKLs

[0504] Isoprene producing *E. coli* strains are constructed to express a phosphoketolase from *Burkholderia phytofirmans* PsJN, *Lactobacillus buchneri* NRRL B-30929, *Bifidobacterium gallicum* DSM 20093, *Bifidobacterium dentium* Bd1, or *Bifidobacterium bifidum* IPLA 20015, or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24). Strains that did not express a phophoketolase are used as controls.

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0505] 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)

[0506] 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*

[0507] Cells expressing the complete MVA pathway and a PKL from *Burkholderia phytofirmans* PsJN, *Lactobacillus buchneri* NRRL B-30929, *Bifidobacterium gallicum* DSM 20093, *Bifidobacterium dentium* Bd1, or *Bifidobacterium bifidum* IPLA 20015, or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24) 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 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. After 3.5 more hours, 1.5 ml sample is centrifuged, the supernatant is discarded and the pellet is resuspended in 100 uL dry-ice cold methanol.

(iii) Intracellular acetyl-phosphate determination.

[0508] To extract acetyl-phosphate, 1.5 mL of E.coli cells grown to OD 0.57-2.26 is spun down by centrifugation and 100 μL of dry-ice cold methanol is added to the pellets. Methanol-quenched samples are stored at -20°C for several days. Further sample processing includs gentle cell re-suspension, 5-min centrifugation at -9°C and aspiration of the supernatant into clean vials. The pellet is re-extracted twice with 75 μL of water containing 2% acetic acid. After each extraction, cell debris are pelleted by centrifugation at -9°C, the supernatants from all three extractions are pooled together and spiked with 1 μL of tributylamine. Mass spectrometric analysis of acetyl phosphate by LCMS is carried out using a Thermo Finnigan TSQ system (Thermo Electron Corporation, San Jose, CA). The system control, data acquisition, and mass spectral data evaluation are performed using XCalibur and LCQuan software (Thermo Electron Corp). A mobile phase gradient is 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 is 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 is 15mM tributylamine/10 mM acetic acid in water, solvent B is methanol, and solvent C is water. Mass detection of acetyl phosphate is 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 is 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). A calibration curve obtained by injection of acetyl phosphate standard (Sigma-Aldrich) is used to calculate concentration of the metabolite in cell extracts. Intracellular concentration of acetyl phosphate is determined based on the assumption that in 1 mL of the culture at OD=200 the integrated volume of all cells is 50 Ml. Produced acetyl phosphate is assessed in strains expressing a PKL as compared to control strain not expressing phosphoketolase.

Example 9: Production of isoprene in recombinant host cells expressing phosphoketolase at small scale

[0509] Isoprene producing *E. coli* strains are constructed to express a phosphoketolase from Burkholderia phytofirmans PsJN, Lactobacillus buchneri NRRL B-30929, Bifidobacterium gallicum DSM 20093, Bifidobacterium dentium Bd1, or Bifidobacterium bifidum IPLA 20015,

or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24), the complete MVA pathway and an isoprene synthase. Isoprene producing strains that did not express a phophoketolase are used as controls.

TM3 media recipe (per liter fermentation media):

[0510] 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)

[0511] 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*

[0512] 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. A 100 µl sample of whole broth is placed in a 96-well glass block. The glass block is 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 is kept at 70°C water bath for 2 minutes and levels of isoprene in the headspace measurement are determined using gas chromatography-mass spectrometry. 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 10: Production of isoprene in recombinant host cells expressing phosphoketolase at 15-L scale

[0513] Isoprene producing *E. coli* strains are constructed to express a phosphoketolase from Burkholderia phytofirmans PsJN, Lactobacillus buchneri NRRL B-30929, Bifidobacterium gallicum DSM 20093, Bifidobacterium dentium Bd1, or Bifidobacterium bifidum IPLA 20015, or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24), the complete MVA pathway and an isoprene synthase. Isoprene producing strains that did not express a phophoketolase are used as controls in a 15 Liter scale experiment for production of isoprene.

(i) *Materials*

Medium Recipe (per liter fermentation medium):

[0514] 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 are added together and dissolved in Di H2O. This solution is heat sterilized (123°C for 20 minutes). The pH is adjusted to 7.0 with ammonium hydroxide (28%) and q.s. to volume. Glucose 10 g, Vitamin Solution 8 mL, and antibiotics are added after sterilization and pH adjustment.

1000X Modified Trace Metal Solution (per liter):

[0515] 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):

[0516] 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):

[0517] 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):

[0518] 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) Analysis

[0519] 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._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 are 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.

Example 11: Production of amorphadiene or farnesene in strains expressing an identified phosphoketolase

[0520] Isoprenoid producing *E. coli* strains are constructed to express a phosphoketolase from Burkholderia phytofirmans PsJN, Lactobacillus buchneri NRRL B-30929, Bifidobacterium gallicum DSM 20093, Bifidobacterium dentium Bd1, or Bifidobacterium bifidum IPLA 20015, or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24), the complete MVA pathway and a codon-optimized gene coding for farnesene synthase or amorphadiene synthase. Isoprenoid producing strains that did not express a phophoketolase are used as controls in an experiment for production of amorphadine or farnesene.

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0521] 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):

[0522] 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*

[0523] 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).

[0524] 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.

Example 12: Construction of phosphoketolase-expressing strains harboring host mutations for producing isoprene.

[0525] Isoprene-producing strains comprising a PKL from Burkholderia phytofirmans PsJN, Lactobacillus buchneri NRRL B-30929, Bifidobacterium gallicum DSM 20093, Bifidobacterium dentium Bd1, or Bifidobacterium bifidum IPLA 20015, or a PKL from Table 1, Table 2, or Clusters 1-22 (see Figures 3-24) 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 38). 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.

[0526] 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 39). 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 13: Production of isoprene by phosphoketolase-expressing strains harboring host mutations at small scale

[0527] The isoprene producing strains described in Example 12 are evaluated for isoprene production at small scale.

(i) *Materials*

TM3 media recipe (per liter fermentation media):

[0528] 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)

[0529] 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*

[0530] 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 14: Production of isoprene by phosphoketolase-expressing strains harboring host mutations at 15-L scale

[0531] The isoprene producing strains described in Example 12 are evaluated for isoprene production at 15-L scale.

(i) *Materials*

Medium Recipe (per liter fermentation medium):

[0532] 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):

[0533] 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):

[0534] 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):

[0535] 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):

[0536] 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) Analysis

[0537] 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. 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 arephytofermans 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.

Example 15: Strains used for Small Scale Evaluation of Phosphoketolases

[0538] The phosphoketolase expressing strains were generated using standard molecular biology techniques where the specified PKL was transformed into MD-891 (BL2 + GI1.2gltA yhfSFRTPyddVIspAyhfS thiFRTtruncIspA pgl ML + FRT-PL.2-3cis-RBS10000-mvk(burtonii) ackA::FRT) together with MCM-1225 (pMCM1225--pCL Ptrc-*E. gallinarum*Upper MVA)). Strains are listed in Table 10.

Table 10. Strains used for Small Scale Evaluation of Phosphoketolases

Source	**PKL #**	**Amino Acid SEQ ID NO:**	**pTrc_IspS_IDI plasmid**	**MD891 strain**	**(MCM1225)**
E. faecium	1	23	MCS811	MCS865	MCS932
L. grayi	2	24	MCS812	MCS866	MCS933
E. casseliflavus	3	25	MCS813	MCS867	MCS934
M. alligatoris	4	26	MCS814	MCS868	MCS935
Carnobacterium	5	27	MCS815	MCS869	MCS936
M. plutonius ATCC	6	28	MCS816	MCS870	MCS937
T. halophilus	7	29	MCS817	MCS871	MCS938
M. plutonius DAT	8	30	MCS818	MCS872	MCS939
M. arthritidis	9	31	MCS819	MCS873	MCS940
S. agalactiae	10	32	MCS820	MCS874	MCS941

M. agalacticae	11	33	MCS821	MCS875	MCS942
S. gordonii	12	34	MCS822	MCS876	MCS943
K. oralis	13	35	MCS823	MCS877	MCS944
M. fermentans	14	36	MCS824	MCS878	MCS945
G. adiacens	15	37	MCS825	MCS879	MCS946
M. hominis	16	38	MCS826	MCS880	MCS947
M. crocodyli	17	39	MCS827	MCS881	MCS948
Neisseria	18	40	MCs828	MCS882	MCS949
E. coleocola	19	41	MCS829	MCS883	MCS950
A. urinae	20	42	MCS830	MCS884	MCS951
K. kingae	21	43	MCS831	MCS885	MCS952
S. criceti (#1)	22	44	MCS832	MCS886	MCS953
S. criceti (#2)	23	45	MCS833	MCS887	MCS954
M. columbinum	24	46	MCS834	MCS888	MCS955
M. gilvum	25	1	MCS835	MCS889	MCS956
S. baltica	26	2	MCs836	MCS890	MCS957
L. rhamnosus	27	3	MCS837	MCS891	MCS958
L. crispatus	28	4	MCS838	MCS892	MCS959
L. citreum	29	6	MCS839	MCS893	MCS960
Bradyrhizobium sp.	30	7	MCS840	MCS894	MCS961
B. microti	31	9	MCS841	MCS895	MCS962
L. salivarius	32	10	MCS842	MCS896	MCS963
R. imtechensis	33	12	MCS843	MCS897	MCS964
B. xenovorans	34	13	MCS844	MCS898	MCS965
M. intracellulare	35	14	MCS845	MCS899	MCS966
Nitrosomonas sp.	36	15	MCS846	MCS900	MCS967
S. pombe	37	16	MCS847	MCS901	MCS968
L. buchneri	38	19	MCS848	MCS902	MCS969
S. ghanaensis	39	20	MCs849	MCS903	MCS970
Cyanothece sp.	40	21	MCS850	MCS904	MCS971
N. fischeri	41	22	MCS851	MCS905	MCS972
L. lactis	42	105	MCS852	MCS906	MCS973
E. gallinarum (CON)		93	EWL1421	MCS908	MCS975

Example 16: *In vivo* screen for phosphoketolase activity in expressing identified Phosphoketolases (PKLs)

[0539] The following *in vivo* screen for phosphoketolase activity was performed as set forth above in Example 7. The host cell background is DW-809 with plasmids pMCS811-pMCS852 containing distinct phosphoketolases.

[0540] For *in vivo* growth evaluation of this set of phosphoketolase (PKL) enzymes, strain DW809, the transketolase double mutant strain as describe in Example 7, was transformed with

plasmids expressing both PKL and isoprene synthase from an IPTG-inducible promoter (see Table 11 for complete list). Individual transformants were identified by growth on M9 glucose minimal medium plates with the aromatic supplement, grown overnight, and then assayed on the Enzyscreen Growth Profiler for growth performance on either glucose or xylose without the aromatic supplement, as described in Example 7. The range of IPTG concentrations used for induction was 0, 20, 40, 60, 80, 100, 200, and 400 μM. To calculate performance index (PI) for growth on glucose or xylose, the OD of each experimental strain was normalized to the OD of the control at a specific time point in the growth curve (typically between 30 and 40 hours). The experimental strains that displayed the highest PIs for growth expressed PKL enzymes with the most preferred *in vivo* activity, whereas the strains with low PIs expressed PKLs that did performed as well in this assay. PIs at 0, 100, and 400 μM were calculated, and were representative of overall growth performance at different induction levels. These are illustrated in Table 11.

Table 11. Performance indices (PI) for growth on glucose or xylose

Source	PKL #	DW-809 strain	PI 0 Glucose	PI 100 Glucose	PI 400 Glucose	PI 0 Xylose	PI 100 Xylose	PI 400 Xylose
E. faecium	1	MCS811	0.55	0.69	0.93	0.84	0.67	0.77
L. grayi	2	MCS812	0.76	0.84	0.67	1.14	0.50	0.45
E. casseliflavus	3	MCS813	4.73	0.50	0.47	2.27	0.77	0.36
M. alligatoris	4	MCS814	4.54	0.66	0.99	1.10	1.11	1.20
Carnobacteriu m	5	MCS815	0.72	0.06	0.06	0.49	0.09	0.17
M. plutonius ATCC	6	MCS816	0.13	0.30	0.11	0.47	0.16	0.30
T. halophilus	7	MCS817	0.06	0.02	0.03	0.39	0.07	0.05
M. plutonius DAT	8	MCS818	0.17	0.11	0.08	0.85	0.18	0.15
M. arthritidis	9	MCs819	2.26	0.43	0.66	9.52	1.33	0.95
S. agalactiae	10	MCS820	3.23	0.96	0.79	0.64	0.61	0.59
M. agalacticae	11	MCS821	1.26	0.94	1.19	11.47	0.88	0.38
S. gordonii	12	MCS822	3.46	0.55	0.54	2.66	1.08	0.65
K. oralis	13	MCS823	4.39	0.67	0.59	2.49	0.82	0.57
M. fermentans	14	MCS824	1.48	0.34	0.32	5.70	0.12	0.15
G. adiacens	15	MCS825	3.87	0.63	0.65	2.48	0.59	0.49
M. hominis	16	MCS826	1.83	0.92	0.83	18.42	2.79	0.54
M. crocodyli	17	MCS827	0.08	0.05	0.06	0.54	0.08	0.07
Neisseria	18	MCs828	1.30	0.60	0.84	11.47	0.54	0.24
E. coleocola	19	MCS829	0.10	0.08	0.13	1.18	0.09	0.05
A. urinae	20	MCS830	3.79	0.81	0.84	2.75	0.53	1.10

K. kingae	21	MCS831	5.09	0.81	1.06	2.36	0.98	1.28
S. criceti #1	22	MCS832	1.22	0.50	0.52	7.44	0.28	0.61
S. criceti #2	23	MCS833	1.46	1.52	1.44	16.23	0.64	0.30
M. columbinum	24	MCS834	1.47	0.13	0.30	2.96	0.30	0.46
M. gilvum	25	MCS835	0.34	0.08	0.09	1.38	0.10	0.16
S. baltica	26	MCs836	0.11	0.04	0.08	1.17	0.07	0.03
L. rhamnosus	27	MCS837	0.43	0.11	0.13	0.58	0.39	0.70
L. crispatus	28	MCS838	1.02	0.19	0.25	0.37	0.05	0.12
L. citreum	29	MCS839	1.34	0.84	0.67	1.43	0.36	0.75
Bradyrhizobium sp.	30	MCS840	0.38	0.09	0.10	0.39	0.11	0.17
B. microti	31	MCS841	0.38	0.12	0.10	0.64	0.13	0.22
L. salivarius	32	MCS842	0.48	1.33	2.39	1.92	2.20	0.99
R. imtechensis	33	MCS843	0.22	1.36	0.06	0.38	0.01	0.01
B. xenovorans	34	MCS844	0.72	0.25	0.25	0.37	0.07	0.15
M. intracellulare	35	MCS845	0.12	0.03	0.07	0.74	0.11	0.13
Nitrosomonas sp.	36	MCS846	0.08	0.04	0.07	0.71	0.13	0.12
S. pombe	37	MCS847	0.83	0.27	0.23	0.43	0.07	0.12
L. buchneri	38	MCS848	0.75	0.23	0.26	1.63	0.07	0.15
S. ghanaensis	39	MCs849	0.70	0.28	0.00	0.33	0.10	0.12
N. fischeri	41	MCS851	0.32	0.07	0.06	0.54	0.01	0.01
L. lactis	42	MCS852	0.82	0.37	0.06	0.33	0.13	0.14
E. gallinarum (CON)		EWL1421	1.00	1.00	1.00	1.00	1.00	1.00

Example 17: Small Scale Evaluation of Isoprene Yield and Isoprene Specific Productivity in Strains Expressing Phosphoketolase

[0541] The isoprene producing strains described in Example 15 were evaluated for isoprene production at small scale.

(i) *Materials and methods*

[0542] Yeast extract, $MgSO_4$, glucose, IPTG, spectinomycin, and carbenicillin were purchased from Sigma. Aluminum foil seal, 48-well sterile 5 mL block, Breathe Easier sealing membrane, 96-well micro titer plates, and 96-well conical bottom plates were purchased from VWR. 96-well glass blocks were purchased from Zinsser Analytic. Equipment: Agilent 6890 GC equipped with a 5973N mass spectrometer, Eppendorf centrifuge 5417R, Sorvall legend RT.

Growth rate measurement:

[0543] 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.

[0544] 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 with overnight cultures 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 at 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} readings were taken every hour after the IPTG induction for 4 hrs. OD_{600} measurements were determined using a SpectraMax Plus190 (Molecular Devices).

Isoprene yield assay:

[0545] 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 media were inoculated in each well of a 48-well sterile block to a final OD_{600} of 0.2. 10 µL of the inoculated cultures were transferred to 90 µL of TM3 media without glucose or yeast extract and sealed with aluminum foil in a 96-well glass block (Zinsser) and incubated at 34°C and 450 rpm for 24hours. After 24hours, the amount of isoprene in the headspace was measured by GC/MS and amount of glucose left in the media in the media to calculate isoprene yield.

Isoprene specific productivity measurement:

[0546] 100 µl of culture was collected in a 96-well glass block. The glass block was sealed with aluminum foil seal and incubated at 34°C while shaking at 450 rpm for 30 minutes using a Thermomixer (Eppendorf). After 30 minutes, the block was incubated at 70°C water bath for 2 minutes. The glass block was allowed to cool to room temperature and then isoprene in the headspace of the wells was measured by GC/MS.

Glucose measurement:

[0547] Glucose samples were collected by centrifuging 300 µl of cell culture in the 96-well conical bottom plate for 10 min at 4°C, 3000rpm. The supernatant was diluted 10-fold in DI water and the glucose concentration was measured using the described glucose oxidase assay.

Glucose oxidase assay:

[0548] ABTS was solubilized in 50 mM sodium acetate pH 5. Glucose oxidase (GOX) and horse radish peroxidase (HRP) were added to the following concentration: 2.74 mg/ml ABTS (powder), 0.1 U/ml HRP, 1 U/ml GOX. The container was wrapped in tin foil to protect from light and stored up to 7 days at 4°C. The glucose standard was prepared by dissolving glucose in MilliQ water across the desired concentration range (i.e serial 2x dilution from 1 mg/ml). 10 µl of test sample was added (dilute reaction supernatant) and/ or glucose standard to a well of a microtiter plate. 90 µl of the ABTS reagent was added and quickly mixed on a plate mixer. The assay plate was transferred to the plate reader and absorbance was monitored at 420 nm for 3-5 minutes. The data file was exported to Excel. The glucose calibration curve was used to calculate the amount of glucose in each well.

Table 12. Parameters for isoprene detection by GC/MS

GCMS Paramaters:		
Column: ZB-5ms 15m x 0.25mm x 0.25µm		
Oven:		
Ramp (°C/min)	Temperature (°C)	Hold Time (min)
0	37	0.6
Total Run Time:		0.6 minutes
Front Inlet Temperature:		110°C
Split Ratio:		50:1
Flow Rate:		2 mL/min
Injection Volume:		100 µL
MS Mode:		EI
MS Source:		230°C
MS Quadrupole:		150°C
MSD Transfer Line Heater (Aux2):		280°C
SIM Mode:		67 amu

(ii) *Results*

To calculate performance index (PI) for each of: (i) Isoprene Specific Productivity at 2 hours; (ii) Isoprene Specific Productivity at 4 hours; (iii) Growth rate; and (iv) Isoprene yield, each experimental strain was normalized to the specific parameter of the control at a specific time point in the growth curve (typically between 15-24 hours). The experimental strains that displayed PI values greater than 1.0 for these evaluated parameters indicated better performance of the evaluated PKL in this isoprene production assay.

Table 13. PI for each of: (i) Isoprene Specific Productivity at 2 hours; (ii) Isoprene Specific Productivity at 4 hours; (iii) Growth rate; and (iv) Isoprene yield

Source	**PKL #**	**MD891 strain**	**PI S. Prod. 2h(mg/L/h/ OD)-MTP**	**PI S. Prod. 4h (mg/L/h/O D)-MTP**	**PI Growth rate (OD at 5 h)-MTP**	**PI Yield (24 h)-MTP**
E. faecium	1	MCS865	1.29	1.02	1.01	1.07
L. grayi	2	MCS866	1.24	0.75	0.99	0.73
E. casseliflavus	3	MCS867	0.84	0.62	0.88	0.87
M. alligatoris	4	MCS868	1.21	0.99	0.90	1.09
Carnobacterium	5	MCS869	0.82	0.68	1.14	0.50
T. halophilus	7	MCS871	1.21	1.17	1.10	0.99
M. plutonius DAT	8	MCS872	0.00	0.00	0.00	0.00
M. arthritidis	9	MCS873	0.61	0.34	0.72	0.64
S. agalactiae	10	MCS874	1.06	0.93	0.95	1.13
K. oralis	13	MCS877	0.92	0.71	0.86	0.99
M. fermentans	14	MCS878	0.25	0.17	0.48	0.03
G. adiacens	15	MCS879	1.02	0.85	0.86	0.96
M. crocodyli	17	MCS881	0.67	0.42	0.68	1.03
E. coleocola	19	MCS883	0.60	0.51	0.61	0.90
A. urinae	20	MCS884	1.07	1.02	0.89	1.05
S. criceti #1	22	MCS886	1.06	0.83	0.85	0.85
M. columbinum	24	MCS888	0.66	0.31	0.63	0.08
M. gilvum	25	MCS889	1.00	1.02	1.00	0.95
L. rhamnosus	27	MCS891	0.66	0.64	1.00	0.70
L. citreum	29	MCS893	1.17	0.84	1.02	0.86
Bradyrhizobium sp.	30	MCS894	1.10	1.15	1.06	0.98
B. microti	31	MCS895	0.94	0.83	1.04	0.89
R. imtechensis	33	MCS897	0.98	0.90	1.05	0.99
B. xenovorans	34	MCS898	1.09	0.92	1.15	0.87
M. intracellulare	35	MCS899	1.12	0.77	1.05	0.82

Nitrosomonas sp.	36	MCS900	0.64	0.55	1.22	0.63
S. pombe	37	MCS901	0.64	0.63	0.78	0.78
L. buchneri	38	MCS902	0.92	0.74	1.17	0.65
S. ghanaensis	39	MCS903	0.92	0.86	1.05	0.90
Cyanothece sp.	40	MCS904	0.79	0.55	1.06	0.69
N. fischeri	41	MCS905	0.79	0.58	1.19	0.58
L. lactis	42	MCS906	1.02	0.85	1.17	0.72
E. gallinarum (CON)		MCS908	1.00	1.00	1.00	1.00

Example 18: Measurement of intracellular metabolites in strains expressing PKLs

(i) *Materials and methods*

Metabolite extraction:

[0549] The strains used for metabolite analysis were the same strains described in Example 17. Thus, these strains were grown under the growth conditions set forth in Example 17 and samples were taken after 4 hour of growth to determine relative concentrations of selected cellular metabolites 500 uL of cell cultures were collected by centrifugation, the supernatant was discarded, 100 uL of dry-ice-cold methanol was added to the pellets, and the tubes with the pellets were immediately frozen in dry ice and placed into a -80°C refrigerator for storage. To extract metabolites, cell pellets covered with methanol were resuspended using glass rods, the tubes were centrifuged in microcentrifuge for 5 min and the resulting supernatants were removed and placed into clean tubes. Cell pellets obtained after the first extraction step were resuspended in 40 uL of 50% methanol/10 mM ammonium acetate mix, cell debris were centrifuged and the supernatants were collected and pooled together with the supernatants obtained after the first extraction. This extraction procedure was repeated one more time to ensure more complete removal of metabolites from cell debris.

[0550] During the extraction-centrifugation samples with cells were kept below 4°C to minimize metabolites degradation. Final pooled extracts was mixed and then cleared by centrifugation.

Metabolite measurements:

[0551] Analysis of metabolites was performed by LCMS on a TSQ Quantim triple quadrupole instrument (Thermo Scientific). System control, data acquisition, and data analysis were done

with XCalibur and LCQuan software (Thermo Scientific). 10 uL samples were applied to a C18 Synergi MAX-RP HPLC column (150x2 mm, 4uM, 80A, Phenomenex) equipped with the manufacturer-recommended guard cartridge. The column was eluted with a gradient of 15 mM acetic acid + 10 mM tributylamine in MilliQ-grade water (solvent A) and LCMS-grade methanol from Honeywell, Burdick & Jackson (solvent B). The 22.5 min gradient was as follows: t = 0 min, 5% B; *t* = 2 min, 5% B; *t* =6 min, 10% B; *t* = 12 min, 20% B; *t* = 18 min, 67% B; *t* = 19 min, 99% B; *t*=21 min, 99% B; ; *t* = 21.5 min, 5% B; *t*=22.5 min, 5% B flow rate 0.4mL/min, column temperature 35°C. Mass detection was carried out using electrospray ionization in the negative mode at ESI spray voltage of 3.0-3.5 kV and ion transfer tube temperature of 350°C. The following SRM transitions were selected for metabolites of interest: 259→79 glucose-6-phosphate (G6P), 339→79 for fructose 1,6-bisphosphate, 167 →79 for phosphoenolpyruvate, 275→ 79 for 6-phosphoglycerate, 259 → 79 eV for ribose-5-phosphate, 139 → 79 for acetyl-phosphate, and 199 → 79 for erythrose 4-phosphate. Scan time for each SRM transition was 0.1 s with a scan width set at 0.7 *m/z*. Argon was used as the collision gas at 1.7 mTorr, and the collision energies were optimized to get maximum signal intensities using corresponding standards purchased from Sigma-Aldrich. The same standards were used to verify the retention times of measured metabolites. Peaks with SRM transitions 369→79 were attributed to heptose-bisphosphates. Concentrations of measured metabolites were expressed as signal intensities normalized to optical densities of the cultures during sampling.

(ii) *Results*

[0552] To calculate performance index (PI) for the production of Acetyl-phosphate (AcP), the amount of each metabolite from the respective experimental strain was normalized to the specific parameter of the control at a specific time point in the growth curve (typically between 30 and 40 hours). The experimental strains that displayed PI values greater than 1.0 for these evaluated parameters indicated better performance of the evaluated PKL in this assay.

Table 14. PI for the production of: (i) acetyl-phosphate (AcP)

Source	PKL #	MD891 strain	PI AcP (AU/OD) - MTP
E. faecium	1	MCS865	2.49
L. grayi	2	MCS866	0.94

E. casseliflavus	3	MCS867	2.12
M. alligatoris	4	MCS868	1.75
Carnobacterium	5	MCS869	0.35
M. plutonius ATCC	6	MCS870	0.00
T. halophilus	7	MCS871	0.48
M. plutonius DAT	8	MCS872	0.00
M. arthritidis	9	MCS873	1.51
S. agalactiae	10	MCS874	1.06
M. agalacticae	11	MCS875	0.00
S. gordonii	12	MCS876	0.00
K. oralis	13	MCS877	2.26
M. fermentans	14	MCS878	0.54
G. adiacens	15	MCS879	1.47
M. hominis	16	MCS880	0.00
M. crocodyli	17	MCS881	1.71
Neisseria	18	MCS882	0.00
E. coleocola	19	MCS883	2.93
A. urinae	20	MCS884	0.98
K. kingae	21	MCS885	0.00
S. criceti #1	22	MCS886	1.31
S. criceti #2	23	MCS887	0.00
M. columbinum	24	MCS888	0.73
M. gilvum	25	MCS889	0.52
S. baltica	26	MCS890	0.00
L. rhamnosus	27	MCS891	2.35
L. crispatus	28	MCS892	0.00
L. citreum	29	MCS893	0.76
Bradyrhizobium sp.	30	MCS894	0.19
B. microti	31	MCS895	0.31
L. salivarius	32	MCS896	0.00
R. imtechensis	33	MCS897	0.19
B. xenovorans	34	MCS898	0.16
M. intracellulare	35	MCS899	0.40
Nitrosomonas sp.	36	MCS900	0.33
S. pombe	37	MCS901	0.19
L. buchneri	38	MCS902	0.19
S. ghanaensis	39	MCS903	0.76
Cyanothece sp.	40	MCS904	0.15
N. fischeri	41	MCS905	0.15
L. lactis	42	MCS906	0.19
E. gallinarum (CON)		MCS908	1.00

Example 19: Determination of Protein Expression and Solubility of Phosphoketolases

(i) *Materials and Methods*

[0553] The strains used to determine protein expression and solubility of the evaluated phosphoketolases were the same strains described in Example 17. The strains were grown in LB broth overnight at 34C with appropriate antibiotics. The next day, 100 uL of the overnight culture was added to 5 mL of LB with appropriate antibiotics and grown at 34C to an OD(600) of ~0.5. The cultures were then induced with 200 uM IPTG and incubated for an additional 6 hours at 34C. The cells were then harvested by centrifugation, and the pellets were stored at -80C.

[0554] The next day the pellets were allowed to thaw, and they were resuspended to an OD(600) of 4 in 100 mM Tris 100 mM NaCl pH 7.6 with 0.2 mg/ml DNaseI and 0.5 mM AEBSF. The cells were then individually lysed via French-press, and the cell debris was removed by centrifugation. The average total protein concentration of the soluble fraction was 0.56 ± 0.22 mg/ml as determined by the standard Bradford assay. The pellet from centrifugation was resuspended in 100 mM Tris 100 mM NaCl pH 7.6 buffer and saved to determine the percent solubility of each phosphoketolase.

[0555] The lysate was then used to determine the amount phosphoketolase (PKL) activity on fructose 6-phosphate (F6P) per unit total protein (μmol/min/mg). The PKL activity on F6P was determined by following the amount of acetyl-phosphate (AcP) generated. The reaction mixture (200 uL) contained 10 mM MgCl2, 10 mM potassium phosphate (pH 7.6), 1 mM thiamine diphosphate, 10 mM F6P, 20 mM NaF, 8 mM iodoacetomide, 1 mM dithiothreitol in 100 mM Tris 100 mM NaCl pH 7.6 with 100 uL of lysate. These incubated for 30 minutes at 34C and were quenched by adding 60 uL of the reaction mixture to 60 uL of 2 M hydroxylamine pH 6.5. This quenched mixture incubated at room temperature for 10 minutes, and then 40 uL of 15% TCA, 40 uL of 4 M HCl and 40 uL of 5% FeCl3 in 0.1 M HCl was added. This final mixture was then centrifuged at 3000 rpm for 5 min. The supernatant (200 uL) was removed, and the absorbance was measured at 505 nm. A calibration curve of AcP was used to calculate how much AcP was produced.

[0556] Relative expression and solubility of each PKL variant, relative to the E. gallinarum MCS908 control, was determined by densitometry. The soluble lysates of each sample were mixed 1:1 with gel loading dye and ran on SDS-PAGE gels. Each pellet, obtained from sample centrifugation post lysis via the French press (see above), was diluted 1:1 with gel loading dye and loaded on SDS-PAGE gels. A sample of E. gallinarum MCS908 soluble lysate was included on each gel as a control. Gels were developed using Coomassie Brilliant Blue stain, and analyzed using ImageQuantTL v2005 (GE Health Sciences) densitometry software. The percent of soluble protein expressed and the percent soluble to insoluble were determined relative to the control strain (E. gallinarum MCS908).

(ii) *Results*

[0557] To calculate performance index (PI) for each of: (i) (F6P) Specific Activity per unit total protein (µmol/min/mg); (ii) Expression level; and (iii) Solubility each experimental strain was normalized to the specific parameter of the control. The PI for F6P Specific Activity (Activity/Expression level) was determined by dividing the PI values for (i) by the PI value to (ii). The experimental strains that displayed a PI greater than 1.0 for these evaluated parameters indicated better performance of the evaluated PKL in this assay.

Table 15. Solubility and expression of each PKL

Source	MD891 strain	PI F6P S.A. (µmol/min/ mg total protein)	PI Expression level (% relative to control)	PI Solubility (% Soluble)	PI F6P Specific Activity (Activity/Expressio n level)
L. grayi	MCS866	1.22	0.33	0.30	3.69
E. casseliflavus	MCS867	2.41	2.35	0.97	1.02
M. alligatoris	MCS868	0.79	0.26	0.77	3.05
Carnobacterium	MCS869	0.10	0.09	0.07	1.14
T. halophilus	MCS871	0.15	0.05	0.16	3.06
M. arthritidis	MCS873	2.52	1.78	0.87	1.41
S. agalactiae	MCS874	1.25	0.49	0.82	2.56
K. oralis	MCS877	2.29	1.81	0.96	1.26
M. fermentans	MCS878	0.29	0.21	0.79	1.38
G. adiacens	MCS879	1.87	1.04	0.99	1.79
M. crocodyli	MCS881	2.21	1.16	0.61	1.90
E. coleocola	MCS883	3.18	1.67	0.95	1.90
A. urinae	MCS884	1.96	1.40	0.98	1.40
M. columbinum	MCS888	1.77	1.75	1.06	1.01

M. gilvum	MCS889	0.65	0.33	0.40	1.96
L. citreum	MCS893	0.90	1.13	1.03	0.80
Bradyrhizobium sp.	MCS894	0.11	0.10	0.29	1.10
B. microti	MCS895	0.42	0.25	0.90	1.69
R. imtechensis	MCS897	0.14	0.06	0.29	2.28
B. xenovorans	MCS898	0.23	0.33	0.22	0.69
M. intracellulare	MCS899	0.14	0.32	0.45	0.43
Nitrosomonas sp.	MCS900	0.22	0.10	0.13	2.23
S. pombe	MCS901	0.16	0.49	0.18	0.32
L. buchneri	MCS902	0.06	0.06	0.08	0.97
S. ghanaensis	MCS903	0.67	0.46	0.23	1.45
Cyanothece sp.	MCS904	1.23	0.48	0.77	2.56
N. fischeri	MCS905	0.07	0.44	0.27	0.15
L. lactis	MCS906	0.23	0.07	0.07	3.24
E. gallinarum (CON)	MCS908	1.00	1.00	1.00	1.00

Example 20: Phosphoketolase activity on fructose 6-phosphate and xylulose 5-phosphate

[0558] This example determined PKL activity when strains are grown on fructose 6-phosphate (F6P) or xylulose 5-phosphate (X5P).

(i) *Materials and methods*

[0559] The strains were grown in LB broth overnight at 34C with appropriate antibiotics. The next day, 200 uL of the overnight culture was added to 5 mL of TM3 with appropriate antibiotics and grown at 34C for 2.5 hours. The cultures were then induced with 200 uM IPTG and incubated for an additional 4 hours at 34C. The cells were then harvested by centrifugation, and the pellets were stored at -80C.

[0560] The next day the pellets were allowed to thaw, and they were resuspended in 2 mL of 100 mM HEPES pH 7.8 with 0.2 mg/ml DNaseI and 0.5 mM AEBSF. The cells were then individually lysed via French-press, and the cell debris was removed by centrifugation.

Lysate Preparation and Enzyme Activity Determination:

[0561] The lysate was then used to determine the amount phosphoketolase (PKL) activity on fructose 6-phosphate (F6P) and xylulose 5-phosphate (X5P). The PKL activity on F6P and X5P

was determined by following the amount of acetyl-phosphate (AcP) generated. The F6P reaction mixture (200 uL) contained 10 mM MgCl2, 10 mM potassium phosphate (pH 7.6), 1 mM thiamine diphosphate, 10 mM F6P, 20 mM NaF, 8 mM iodoacetomide, 1 mM dithiothreitol in 100 mM HEPES pH 7.8 with and 100 uL of lysate. These incubated for 30 minutes at 34C and were quenched by adding 60 uL of the reaction mixture to 60 uL of 2 M hydroxylamine pH 6.5. This quenched mixture incubated at room temperature for 10 minutes, and then 40 uL of 15% TCA, 40 uL of 4 M HCl and 40 uL of 5% FeCl3 in 0.1 M HCl was added. This final mixture was then centrifuged at 3000 rpm for 5 min. The supernatant (200 uL) was removed, and the absorbance was measured at 505 nm. A calibration curve of AcP was used to calculate how much AcP was produced. The X5P activity was measured with a similar method. The X5P reaction mixture (200 uL) contained 10 mM MgCl2, 10 mM potassium phosphate (pH 7.6), 1 mM thiamine diphosphate, 10 mM ribose 5-phosphate, 60 ug/mL of ribulose-5-phosphate 3-epimerase, 200 ug/mL of ribose-5-phosphate isomerase A, 20 mM NaF, 8 mM iodoacetomide, 1 mM dithiothreitol in 100 mM HEPES pH 7.8 with and 20 uL of lysate. Due to the wide range of activities on X5P, the activities were measured at two concentrations of lysate: undiluted and five-fold diluted into 100 mM HEPES pH 7.8.

Table 16. PKL activity on F6P or X5P

	F6P		**X5P (Undiluted)**		**X5P (Diluted 5X)**			
Strain Description (MD-891 Strain)	**AcP (mM)**	**Spec Act**	**AcP (mM)**	**Spec Act**	**AcP (mM)**	**Spec Act**	**Ratio Undiluted (X/F)**	**Ratio Diluted (X/F)**
pMCS842, pMCM1225	0.51	0.19	1.75	3.21	0.41	3.79	17.03	20.16
pMCS836, pMCM1225	0.04	0.04	0.11	0.54	0.085	2.10	15.19	59.62
pEWL1421, pMCM1225	1.19	0.65	3.59	9.74	0.76	10.37	15.06	16.04
pMCS813, pMCM1225	1.99	1.06	4.97	13.19	1.26	16.76	12.49	15.88
pMCS821, pMCM1225	1.98	1.20	4.82	14.59	1.27	19.16	12.20	16.022
pMCS833, pMCM1225	2.02	1.14	4.70	13.28	1.12	15.83	11.68	13.91
pMCS830, pMCM1225	1.45	0.63	3.10	6.73	0.81	8.77	10.67	13.90
pMCS822 , pMCM1225	1.52	1.27	3.23	13.48	0.67	13.86	10.61	10.91
pMCS839, pMCM1225	0.37	0.37	0.77	3.85	0.19	4.88	10.45	13.25
pMCS825, pMCM1225	2.02	1.26	4.14	12.92	1.16	18.08	10.22	14.30
pMCS823, pMCM1225	2.51	1.02	4.65	9.48	1.25	12.70	9.28	12.43
pMCS826, pMCM1225	2.43	1.81	4.36	16.19	0.94	17.50	8.95	9.68
pMCS824, pMCM1225	0.37	6.81	0.64	59.24	0.19	89.62	8.69	13.15
pMCS834, pMCM1225	2.04	1.16	3.39	9.61	0.87	12.30	8.32	10.64

pMCS811, pMCM1225	0.87	0.33	1.45	2.80	0.39	3.79	8.31	11.24
pMCS819, pMCM1225	3.53	1.85	5.13	13.45	1.34	17.65	7.26	9.52
pMCS820, pMCM1225	0.81	0.32	1.16	2.26	0.32	3.12	7.18	9.91
pMCS838, pMCM1225	0.24	0.15	0.34	1.06	0.16	2.49	7.05	16.62
pMCS829 , pMCM1225	3.30	2.15	4.45	14.46	1.16	18.93	6.73	8.81
pMCS832, pMCM1225	2.10	1.78	2.76	11.73	0.69	14.62	6.59	8.21
pMCS827 , pMCM1225	1.33	2.28	1.73	14.84	0.36	15.45	6.51	6.78
pMCS831, pMCM1225	0.78	0.64	0.99	4.08	0.23	4.63	6.41	7.282
pMCS828, pMCM1225	2.62	2.32	3.30	14.62	0.87	19.25	6.31	8.31
pMCS845, pMCM1225	0.19	0.12	0.17	0.53	0.09	1.40	4.36	11.52
pMCS814, pMCM1225	0.54	0.30	0.37	1.01	0.17	2.37	3.36	7.87
pMCS844, pMCM1225	0.21	0.16	0.12	0.47	0.09	1.79	2.89	11.07
pMCS816, pMCM1225	0.19	0.11	0.11	0.33	0.12	1.74	2.87	15.25
pMCS849, pMCM1225	0.82	0.38	0.47	1.08	0.14	1.62	2.83	4.23
pMCS645, pMCM1225	0.22	0.20	0.13	0.56	0.10	2.31	2.81	11.57
pMCS818, pMCM1225	0.18	0.19	0.09	0.48	0.10	2.67	2.54	13.99
pMCS841, pMCM1225	0.78	0.45	0.37	1.09	0.11	1.66	2.42	3.69
pMCS837, pMCM1225	- 0.07	-0.03	0.61	1.17	0.10	1.01	- 43.14	-37.25

Example 21: 14L Evaluation of Isoprene Production in Strains Expressing Phosphoketolase

[0562] This experiment was performed to evaluate the effect of expressing various phosphoketolase enzymes on isoprene production. All the strains in this experiment used a modified *E. coli* host (BL21 derived production host MD891) which expresses introduced genes from the mevalonate pathway, isoprene synthase and phosphoketolase (PKL), for strain details see Table 17. All of these isoprene producing strains were grown in fed-batch culture at the 15-L scale.

[0563] The relevant performance metrics are cumulative isoprene yield on glucose, and isoprene titer. The productivity metrics are found summarized in Table 18.

Table 17. List of strains

Strain Name	Host	IPTG inducible Upper pathway plasmid	IPTG inducible Isoprene synthase / Phosphoketolase plasmid.
MD13-896	MD-891	pMCM1225	pEWL1418 (PTrc IspS-PKL_B.longum)

MD13-898	MD-891	pMCM1225	pEWL1436 (PTrc IspS-PKL_ C.Acetobutylicum)
MCS674	MD-891	pMCM1225	(PTrc IspS-PKL_ Bifidobacterium bifidum)
MCS675	MD-891	pMCM1225	(PTrc IspS-PKL_ Bifidobacterium dentium)
MCS676	MD-891	pMCM1225	(PTrc IspS-PKL_ Bifidobacterium gallicum)
MCS703	MD-891	pMCM1225	pMCS668 (PTrc IspS-PKL_ E. gallinarum -RBS 2300)
MCS704	MD-891	pMCM1225	pMCS669 (PTrc IspS-PKL_ E. gallinarum -RBS 7700)
MCS706	MD-891	pMCM1225	pMCS671 (PTrc IspS-PKL_ E. gallinarum -RBS 73300)
DW891-2	MD-891	pMCM1225	pMCS822 (PTrc IspS-PKL_ S.gordonii)
DW892-1	MD-891	pMCM1225	pMCS831 (PTrc IspS-PKL_ K.kingae)
MCS935	MD-891	pMCM1225	pMCS814 (PTrc IspS-PKL4[M. alligatoris])
MCS941	MD-891	pMCM1225	pMCS820 (PTrc IspS-PKL10[S. agalacticae])
MCS946	MD-891	pMCM1225	pMCS825 (PTrc IspS-PKL15 [G. adiacens])
MCS699	MD-891	pMCM1225	pMCS666 (PTrc IspS-PKL_E.gallinarum_ RBS2600)
MCS951	MD-891	pMCM1225	pMCS830 (PTrc IspS-PKL_ A.urinae)
MCS944	MD-891	pMCM1225	pMCS823 (PTrc IspS-PKL_ K.oralis)
MCS932	MD-891	pMCM1225	pMCS811 (PTrc IspS-PKL_ E.faecium)
MCS934	MD-891	pMCM1225	pMCS813 (PTrc IspS-PKL3[E. casseliflavus])
MCS963	MD-891	pMCM1225	pMCS842 (PTrc IspS-PKL_ L.salivarus)
MCS947	MD-891	pMCM1225	pMCS826 (PTrc IspS-PKL_ M.hominis)

(i) *Materials and methods*

Medium Recipe (per liter fermentation medium):

[0564] K_2HPO_4 7.5 g, $MgSO_4 * 7H_2O$ 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 H_2O. 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 (spectinomycin and carbenicillin) were added after sterilization and pH adjustment to a target concentration of 50mg/L.

1000X Modified Trace Metal Solution (per liter):

[0565] Citric Acids * H_2O 40 g, $MnSO_4$ * H_2O 30 g, NaCl 10 g, $FeSO_4$ * $7H_2O$ 1 g, $CoCl_2$ * $6H_2O$ 1 g, ZnSO * $7H_2O$ 1 g, $CuSO_4$ * $5H_2O$ 100 mg, H_3BO_3 100 mg, $NaMoO_4$ * $2H_2O$ 100 mg. Each component was dissolved one at a time in Di H_2O, 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):

[0566] 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 H_2O, 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):

[0567] $MgSO_4$ * $7H_2O$ 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):

[0568] 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. For a target of 100µM IPTG: 1.87ml of a sterile 10mg/ml solution is added per kilogram of feed.

[0569] This experiment was carried out to monitor isoprene production from glucose at the desired fermentation pH (7.0) and temperature (34°C). To start each experiment, the appropriate frozen vial of the *E. coli* production 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.

[0570] The inlet gas using to maintain bioreactor backpressure at 0.7 bar gauge and to provide the oxygen to the production organisms was supplied by in house facilities that dilute the inlet gas to a known concentration (7.3 to 8.3 vol% oxygen).

[0571] The batched media had glucose batched in at 9.7 g/L. Induction was achieved by adding isopropyl-beta-D-1-thiogalactopyranoside (IPTG). A syringe containing a sterile solution of IPTG was added to bring the IPTG concentration to 100μM 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. At a fixed time after dissolved oxygen limitation was established, the temperature was raised from 34°C to 37°C over the course of one hour. The fermentation was run long enough to determine the maximum cumulative isoprene mass yield on glucose, typically a total of 64 hrs elapsed fermentation time (EFT).

(ii) *Results and Analysis*

[0572] Isoprene, Oxygen, Nitrogen, and Carbon Dioxide levels in the off-gas were determined independently by a Hiden HPR20 (Hiden Analytical) mass spectrometer.

[0573] Dissolved Oxygen in the fermentation broth is measured by sanitary, sterilizable probe with an optical sensor provided Hamilton Company.

[0574] 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 minute

[0575] Cumulative Isoprene yield on glucose is equal to Isoprene total weight (t)/[(Feed Wt(0)-Feed Wt(t)+83.5)*0.5826)], where 0.5826 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. Units are $g_{isoprene}/g_{glucose}$ * 100, expressed as percentages.

[0576] IspER is the Isoprene Evolution Rate in (mmol/L/hr).

[0577] Specific productivity (mg/L/hr/OD) = IspER*68.117g/mol/OD.

[0578] OD = optical density = Absorbance at 550nm * dilution factor in water.

[0579] Smoothed Specific productivity (mg/L/hr/OD) = slope of milligrams isoprene produced per hour (averaged over 8 hour interval) / broth volume * OD.

[0580] Isoprene titer ($g_{Isoprene}/L_{average\ broth}$) is the total evolved isoprene per average broth volume. It is calculated by integrating the IspER and converting the isoprene unit from mmol to grams.

[0581] The results are depicted graphically in Figures 40 and 41 and illustrated in Table 15.

Table 18. Isoprene Productivity Metrics

Strain Name	PKL (in MD891ackA-host)	Avg EOF Isoprene Titer (g/L)	Avg Cumulative Yield (g/g*100)
DW891-2	*S.gordonii*	104.36	18.20
DW892-1	*K.kingae*	110.32	17.73
MCS944	*K.oralis*	96.11	17.57
MCS932	*E.faecium*	84.76	16.92
MCS946	*G. adiacens*	99.20	16.86
MCS941	*S. agalacticae*	108.37	16.83
MCS674	*B.bifido*	66.12	16.46

MCS951	*A. urinae*	81.77	16.41
MD13-898	*C.acetobutylicum*	85.13	15.90
MCS935	*M. alligatoris*	68.48	15.67
MCS675	*B.dentium*	70.15	15.66
MCS963	*L.salivarus*	83.91	15.32
MCS934	*E.casseliflavus*	80.17	15.07
MD13-896	*B.longum*	85.09	14.74
MCS947	*M.hominis* (decreased IPTG)	59.42	12.04
MCS947	*M.hominis*	17.69	10.84
MCS676	*B.gallicum*	2.12	8.17

Example 22: *In vivo* Evaluation of growth in PKL expressing strains Blocked for Glycolysis and Pentose Phosphate Pathways

[0582] For analysis of PKL enzyme activity in a strain blocked for glycolysis and pentose phosphate pathways, a subset of expression plasmids was transformed into strain MD1041 (HMB GI1.2gltA yhfSFRTPyddVIspAyhfS thiFRTtruncIspA pgl ML, FRT-PL.2-2cis-RBS10000-MVK(burtonii), t zwf::FRT, t pfkA::Frt + t ackA::FRT, t pfkB::Frt) using standard molecular biology techniques. Individual transformants were grown overnight in LB, diluted into TM3 medium with 1% glucose-6-phosphate (Sigma) as the carbon source, and induced with 0, 20, 40, 60, 80, 100, 200, or 400 μM IPTG. Strains were assayed for growth performance on the Enzyscreen Growth Profiler in comparison to MD1041 control strains that did not express any PKL (and therefore did not grow), expressed the PKL enzyme from *E. gallinarum* (and were representative of baseline performance), or WT strains that had no metabolic block in glycolysis or pentose phosphate pathways (as a control for optimal growth).

[0583] To calculate performance index (PI) for growth, MD1041 derivative strains that expressed experimental PKL enzymes were compared to MCS1148, a strain that expressed the PKL from *E. gallinarum* (see Table 1 for strain list). The 35 hour time point and 100 μM IPTG induction level were chosen as representative of general performance throughout the growth curve. To normalize values between assay plates, a correction factor, based on the difference between max OD values of WT strains, of 1.279 was applied to all values in the plate that did not contain the control strain expressing *E. gallinarum* PKL. PI was then calculated by dividing the corrected experimental OD value by the OD value of MCS1148 at the 35 hour time point.

The PI of MCS1148 was therefore 1.0, and any value higher than this indicated an X-fold improvement to growth in this assay. PI values are shown in Table 19.

Table 19: PI values in PKL expressing strains Blocked for Glycolysis and Pentose Phosphate Pathways

Strain	PKL	PI Growth at 35 hours
MD1059	No PKL	0.167
MCS1106	pMCS811(pTrc_IspS_PKL1[E.faecium])	0.606
MCS1108	pMCS813(pTrc_IspS_PKL3[E. casseliflavus])	0.328
MCS1109	pMCS814(pTrc_IspS_PKL4[M. alligatoris])	0.740
MCS1116	pMCS821(pTrc_IspS_PKL11[M. agalacticae])	0.579
MCS1118	pMCS823 (pTrc_IspS_PKL13 [K. oralis])	1.761
MCS1120	pMCS825 (pTrc_IspS_PKL15 [G. adiacens])	0.560
MCS1121	pMCS826 (pTrc_IspS_PKL16 [M. hominis])	0.824
MCS1123	pMCS828 (pTrc_IspS_PKL18 [Neissaria])	0.262
MCS1124	pMCS829 (pTrc_IspS_PKL19 [E. coleocola])	0.164
MCS1125	pMCS830 (pTrc_IspS_PKL20 [A. urinae])	1.090
MCS1126	pMCS831(pTrc_IspS_PKL21 [K. kingae])	0.607
MCS1127	pMCS832(pTrc_IspS_PKL22 [S. criceti #1])	0.099
MCS1128	pMCS833(pTrc_IspS_PKL23 [S. criceti #2])	0.587
MCS1137	pMCS842(pTrc_IspS_PKL32 [L. salivarius])	0.125
MCS1148	pMCS625 (pEWL1421= pTrc_IspS_gallinarumPKL)	1.000
MCS1150	pMCS644 (pTrc_IspS_dentiumPKL)	0.116
MCS1152	pMCS646 (pTrc_IspS_acetobutylicum optimizedPKL)	0.163
MCS1153	pMCS647 (pTrc_IspS_truncatedmMVK; gi1.6_acetobutylicum optimized PKL	1.727
MCS1162	pMCS1008 (pTrc_IspS_PKL-ANC110)	0.239
MCS1168	pMCS1019 (pTrc_IspS_RBS3_PKL16 [M. hominis])	0.120

CLAIMS

What is claimed is:

1. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8.

2. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate.

3. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:8 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity.

4. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:23.

5. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:24.

6. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:25.

7. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:26.

8. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:27.

9. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:28.

10. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:29.

11. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:30.

12. The recombinant cell of any one of claims 1-3, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:31.

13. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11.

14. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said recombinant cell comprising said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) cell growth on glucose, (b) cell growth

on xylose, (c) production of intracellular acetyl-phosphate or (d) cell growth on glucose-6-phosphate.

15. A recombinant cell capable of increased carbon flux through the phosphoketolase pathway, wherein the recombinant cell comprises: (i) a heterologous nucleic acid sequence encoding a polypeptide having phosphoketolase activity, wherein the polypeptide comprises at least 65% sequence identity to SEQ ID NO:11 and (ii) one or more nucleic acids encoding one or more polypeptides of the complete MVA pathway, wherein said polypeptide having phosphoketolase activity of (i) has a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) protein solubility, (b) protein expression, or (c) fructose-6-phosphate (F6P) Specific Activity.

16. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:32.

17. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:33.

18. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:34.

19. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:35.

20. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:36.

21. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:37.

22. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:38.

23. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:39.

24. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:40.

25. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:41.

26. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:42.

27. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:43.

28. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:44.

29. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:45.

30. The recombinant cell of any one of claims 13-15, wherein the polypeptide comprises at least 90% sequence identity to SEQ ID NO:46.

31. The recombinant cell of any one of claims 1-30, wherein culturing of the recombinant cell in a suitable media increases one or more of an intracellular amount of erythrose 4-phosphate, an intracellular amount of glyceraldehyde 3-phosphate, or intracellular amount phosphate.

32. The recombinant cell of any one of claims 1-31, wherein the polypeptide having phosphoketolase activity is capable of synthesizing glyceraldehyde 3-phosphate and acetyl phosphate from xylulose 5-phosphate.

33. The recombinant cell of any one of claims 1-32, wherein the polypeptide having phosphoketolase activity is capable of synthesizing erythrose 4-phosphate and acetyl phosphate from fructose 6-phosphate.

34. The recombinant cell of any one of claims 2-12 or 14-33, wherein the 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.

35. A recombinant cell capable of producing isoprene, wherein the recombinant cell of any one of claims 1-30 further comprises a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein culturing of the recombinant cell in a suitable media provides for the production of isoprene with a Performance Index value of greater than 1.0 in one or more of the following parameters: (a) isoprene yield or (b) isoprene specific productivity.

36. The recombinant cell of claim 35, wherein the heterologous nucleic acid encoding an isoprene synthase polypeptide is a plant isoprene synthase polypeptide.

37. The recombinant cell of claim 36, wherein the plant isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba x Populus tremula.*

38. The recombinant cell of claim 36, wherein the isoprene synthase polypeptide is selected from the group consisting of *Pueraria montana, Pueraria lobata, Populus tremuloides, Populus alba, Populus nigra,* and *Populus trichocarpa.*

39. The recombinant cell of any one of claims 34-38, wherein the recombinant cells further comprise one or more nucleic acids encoding one or more 1-deoxy-D-xylulose 5-phosphate (DXP) pathway polypeptides.

40. A recombinant cell capable of producing isoprenoid precursors, wherein the recombinant cell of any one of claims 1-34 is cultured in a suitable media and produces said isoprenoid precursors.

41. A recombinant cell capable of producing isoprenoids, wherein the recombinant cell of any one of claims 1-34 further comprises a heterologous nucleic acid encoding an polyprenyl pyrophosphate synthase polypeptide, wherein culturing of the recombinant cell in a suitable media provides for the production of isoprenoids.

42. A recombinant cell capable of producing an acetyl CoA-derived metabolite, wherein culturing of the recombinant cell of any one of claims 1-34 in a suitable media provides for the production of the acetyl CoA-derived metabolite.

43. The recombinant cell of any one of claims 1-42, wherein the nucleic acid is placed under an inducible promoter or a constitutive promoter.

44. The recombinant cell of any one of claims 1-42, wherein the nucleic acid is cloned into one or more multicopy plasmids.

45. The recombinant cell of any one of claims 1-42, wherein the nucleic acid is integrated into a chromosome of the cells.

46. The recombinant cell of any one of claims 1-45, wherein the recombinant cells are gram-positive bacterial cells, gram-negative bacterial cells, fungal cells, filamentous fungal cells, algal cells or yeast cells.

47. The recombinant cell of any one of claims 1-46, wherein the recombinant cells are selected from the group consisting of *Corynebacteria, Bacillus subtilis, Streptomyces lividans, Streptomyces coelicolor, Streptomyces griseus, Escherichia coli, Pantoea citrea, Trichoderma reesei, Aspergillus oryzae and Aspergillus niger, Saccharomyces cerevisiae* and *Yarrowia lipolytica*.

48. The recombinant cell of claim 41, wherein the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpene, and polyterpene.

49. The recombinant cell of claim 41, wherein the isoprenoid is a sesquiterpene.

50. The recombinant cell of claim 41, wherein the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

51. The recombinant cell of claim 42, wherein the acetyl CoA-derived metabolite is selected from the group consisting of polyketides, polyhydroxybutyrate, fatty alcohols, and fatty acids.

52. The recombinant cell of claim 42, wherein the acetyl CoA-derived metabolite is selected from the group consisting of glutamic acid, glutamine, aspartate, asparagine, proline, arginine, methionine, threonine, cysteine, succinate, lysine, leucine, and isoleucine.

53. The recombinant cell of claim 42, wherein the acetyl CoA-derived metabolite is selected from the group consisting of acetone, isopropanol, isobutene, and propene.

54. The recombinant cell of any one of claims 35 and 40-42, wherein the suitable media comprises a carbon source.

55. The recombinant cell of claim 54, wherein the carbon source is a carbohydrate selected from the group consisting of monosaccharide, disaccharide, oligosaccharide, polysaccharide, C6 sugar, C5 sugar, and invert sugar.

56. A method of producing isoprene comprising: (a) culturing the recombinant cell of claim 35 under conditions suitable for producing isoprene and (b) producing isoprene.

57. A method of producing an isoprenoid precursor comprising: (a) culturing the recombinant cell of claim 40 under conditions suitable for producing an isoprenoid precursor and (b) producing an isoprenoid precursor.

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

59. A method of producing an acetyl CoA-derived metabolite comprising: (a) culturing the recombinant cell of claim 42 under conditions suitable for producing an acetyl CoA-derived metabolite and (b) producing an acetyl CoA-derived metabolite.

60. A method for detecting in vivo phosphoketolase activity of a polypeptide in a recombinant cell comprising (a) culturing a recombinant cell comprising a heterologous nucleic acid sequence encoding said polypeptide wherein the recombinant cell is defective in transketolase activity (tktAB) under culture conditions with glucose or xylose as a carbon source; (b) assessing cell growth of the recombinant cell and (c) detecting in vivo phosphoketolase activity of said polypeptide based upon the presence of cell growth.

61. An isolated polypeptide with phosphoketolase activity detected by the method of claim 60.