converts acetaldehyde directly to acetyl-CoA without expenditure of ATP, and reduces one NAD^+ to one NADH in the process.

[0009] While the ATP savings gained from replacement of the PDH-bypass with ADA can be utilized towards higher product yields, there are potential shortcomings associated with the use of ADA in combination with the native mevalonate pathway. First, inactivation of the native PDH-bypass removes one source of NADPH, while the reaction catalyzed by ADA produces NADH. Thus, the replacement of the PDH-bypass with ADA, without further pathway modification, introduces a redox imbalance in isoprenoid synthesis, which consumes NADPH.

[0010] Secondly, ADA catalyzes the following reversible reaction:

$$\text{Acetaldehyde} + NAD^+ + \text{Coenzyme A} \Leftrightarrow \text{Acetyl-CoA} + NADH + H^+$$

The native PDH-bypass reaction for forming acetyl-CoA is thermodynamically favorable because the reaction is coupled to the hydrolysis of ATP to AMP. In contrast, the ADA reaction is not coupled to ATP, and is much closer to equilibrium than the native PDH-bypass reactions for forming Acetyl-CoA. Thus, the reaction catalyzed by ADA has a lower ~~a~~ thermodynamic driving force behind the conversion of acetaldehyde to acetyl-CoA, and without further pathway modification, the theoretical energy gains of ADA may not be realized.

[0011] The compositions and methods described herein address these shortcomings. In some ~~embodiments~~examples, to address the redox imbalance introduced by replacement of the PDH-bypass with ADA, the genetically modified host cells further utilize an NADH-using enzyme in the isoprenoid pathway to consume ADA-generated NADH. Thus, the pool of NADH generated by the ADA-mediated conversion of acetaldehyde to acetyl-CoA can be utilized directly towards isoprenoid synthesis. In some ~~embodiments~~examples, the NADH-using enzyme is an enzyme that is non-native to the isoprenoid pathway. For example, the NADH-using enzyme can replace an NADPH-using enzyme that is native to the isoprenoid pathway. In particular ~~embodiments~~examples, the NADH-using enzyme is an NADH-using 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) that converts HMG-CoA to mevalonate.

[0012] In some ~~embodiments~~examples, to address the lower thermodynamic driving force behind the ADA reaction, the genetically modified host cells further utilize, as a first step in the mevalonate pathway, a thermodynamically favorable reaction immediately downstream of acetyl-CoA to provide a pull on the ADA reaction. In some ~~embodiments~~examples, the formation of acetoacetyl-CoA from acetyl-CoA is catalyzed by an acetoacetyl-CoA synthase (AACS;

alternately referred to as an acetyl-CoA:malonyl-CoA acyltransferase). The reaction catalyzed by AACS is thermodynamically more favorable than the reaction catalyzed by the acetyl-CoA thiolase of the native mevalonate pathway, due to the hydrolysis of 1 ATP resulting from the generation of malonyl-CoA by acetyl-CoA carboxylase (**FIG. 5**). Thus, AACS provides a stronger pull on acetyl-CoA to drive the ADA reaction forward.

[0013] The advantages of utilizing a heterologous ADA in combination with these modifications are exemplified by the improved theoretical yield of the sesquiterpene farnesene in host cells comprising a MEV pathway. Isoprenoid production via the native mevalonate pathway is illustrated in **FIG. 1** and **FIG. 2.** As indicated in **FIG. 3**, when cytosolic acetyl-CoA is synthesized from glucose using only the chemical reactions which occur in the native yeast metabolic network, the maximum possible stoichiometric yield for conversion of glucose to farnesene via the mevalonate pathway is 23.6 wt%, with 4.77 molecules of glucose being required for the synthesis of each molecule of farnesene. 27 ATP are required per molecule of farnesene, 18 of which are consumed in the synthesis of cytosolic acetyl-CoA from acetaldehyde via the PDH-bypass. However, by including the reactions catalyzed by ADA and NADH-using HMG-CoA reductase into the metabolic network for mevalonate production, as illustrated in **FIG. 4**, the maximum theoretical stoichiometric yield is improved to 25.2 wt%. In particular, ADA converts acetaldehyde to acetyl-CoA without any ATP input; this reduces the ATP equivalents required for farnesene synthesis to 9, resulting in a savings of 18 ATP equivalents per molecule of farnesene produced (2 ATP equivalents per acetyl-CoA x 9 acetyl-CoAs per 1 farnesene). ~~This~~These savings in ATP usage during acetyl-CoA production eliminates the cell's need for oxygen to run the TCA cycle for farnesene production. The oxygen requirement for conversion of glucose to farnesene decreases from 7.8 molecules of O_2 per glucose consumed to 6, thereby reducing a major production cost of providing oxygen to fermenters at scale. In addition, redox imbalance is alleviated by co-introduction of an NADH-using HMG-CoA reductase, which consumes NADH generated by ADA.

[0014] As indicated in **FIG. 4**, there remains a stoichiometric excess of ATP in a strain that comprises both an ADA and an NADH-using HMG-CoA reductase, which can be used by the cell for maintenance and growth. Alternatively, some of this excess ATP can be utilized towards improving the kinetics of acetoacetyl-CoA production, by introducing an acetoacetyl-CoA synthase (AACS). As illustrated in **FIG. 5**, AACS is an enzyme which synthesizes acetoacteyl-CoA from malonyl-CoA and acetyl-CoA. Malonyl-CoA synthesis requires an energetic input of 1 ATP per molecule of acetyl-CoA converted (catalyzed by

acetyl-CoA carboxylase, thereby improving the thermodynamic driving force of acetoacetyl-CoA synthesis from acetyl-CoA. Importantly, this does not affect the maximum stoichiometric yield of farnesene from sugar or the oxygen demand of the pathway, as there is still excess ATP available in this strain design, as illustrated in **FIG. 6**.

[0015] As shown in **FIG. 7**, additional efficiencies can be gained via the introduction of phosphoketolase (PK) and phosphotransacetylase (PTA) enzymes. PK and PTA catalyze the reactions to convert fructose-6-phosphate (F6P) or xyulose-5-phosphate (X5P) to acetyl-CoA. With these metabolic pathways available, at optimality, the reaction network is able to reach 29.8 wt% mass yield or greater, a significant increase in maximum theoretical yield. This solution involves diverting carbon away from lower glycolysis (G3P → pyruvate), which results in less ATP and NADH generation, both of which are already in excess in a network comprising the ADA and NADH-using HMG-CoA reductase modifications. One benefit of reducing flux through lower glycolysis is that less CO_2 is produced in converting pyruvate into acetaldehyde, and thus more carbon can be captured in the end product, thereby increasing the maximum theoretical yield of the network. A second benefit is that less NADH is produced, and therefore significantly less oxygen is needed to reoxidize it. In particular, the oxygen demand at optimality is only 1.84 molecules of O_2 per glucose consumed. The redox impact of the addition of PK and PTA to an ADA background is visible even at low yields in the microscale, as illustrated in **FIG. 13,** where glycerol production returns to wild-type levels.

[0016] Thus, provided herein are genetically modified host cells and methods of their use for the production of acetyl-CoA-derived isoprenoids. In one aspect, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate; and (b) a heterologous nucleic acid encoding an acylating acetylaldehyde dehydrogenase.

[0017] In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an acetyl-CoA:malonyl-CoA acyltransferase (*i.e.*, an acetoacetyl-CoA synthase (AACS)).

[0018] In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an NADH-using enzyme that converts HMG-CoA to mevalonate. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase.

[0019] In some ~~embodiments~~examples, the genetically modified host cell further comprises a heterologous nucleic acid encoding a phosphoketolase. In some ~~embodiments~~examples, the genetically modified host cell further comprises a heterologous nucleic acid encoding a phosphotransacetylase.

[0020] In some ~~embodiments~~examples, the amino acid sequence of the ADA is at least 80% identical to SEQ ID NO:2. In some ~~embodiments~~examples, the amino acid sequence of the acetyl-CoA:malonyl-CoA acyltransferase is at least 80% identical to SEQ ID NO:16. In some ~~embodiments~~examples, the amino acid sequence of the NADH-using HMG-CoA reductase is at least 80% identical to SEQ ID NO:20. In some ~~embodiments~~examples, the amino acid sequence of the phosphoketolase is at least 80% identical to SEQ ID NO:12. In some ~~embodiments~~examples, the amino acid sequence of the phosphotransacetylase is at least 80% identical to SEQ ID NO:14.

[0021] In some ~~embodiments~~examples, the genetically modified host cell further comprises a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH)-bypass. In some ~~embodiments~~examples, the one or more enzymes of the PDH-bypass are selected from acetyl-CoA synthase 1 (ACS1), acetyl-CoA synthase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6). In some ~~embodiments~~examples, ACS1 is functionally disrupted. In some ~~embodiments~~examples, ACS2 is functionally disrupted. In some ~~embodiments~~examples, ALD6 is functionally disrupted. In some ~~embodiments~~examples, ACS1 and ACS2 are functionally disrupted. In some ~~embodiments~~examples, ACS1, ACS2 and ALD6 are functionally disrupted.

[0022] In some ~~embodiments~~examples, the genetically modified host cell further comprises a functional disruption of one or more enzymes having alcohol dehydrogenase (ADH) activity. In some ~~embodiments~~examples, the one or more enzymes having ADH activity are selected from alcohol dehydrogenase 1 (ADH1), alcohol dehydrogenase 3 (ADH3), alcohol dehydrogenase 4 (ADH4), and alcohol dehydrogenase 5 (ADH5).

[0023] In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-

pyrophosphate to isopentenyl pyrophosphate. In some ~~embodiments~~examples, the one or more enzymes of the MEV pathway are selected from HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase.

[0024] In some ~~embodiments~~examples, the host cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway. In some ~~embodiments~~examples, the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of a single transcriptional regulator. In some ~~embodiments~~examples, the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of multiple heterologous transcriptional regulators.

[0025] In some ~~embodiments~~examples, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP). In some ~~embodiments~~examples, the genetically modified host cell further comprises a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound. In some ~~embodiments~~examples, the genetically modified host cell further comprise a heterologous nucleic acid encoding an enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound. In some ~~embodiments~~examples, the enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound is selected from the group consisting of carene synthase, geraniol synthase, linalool synthase, limonene synthase, myrcene synthase, ocimene synthase, α-pinene synthase, β-pinene synthase, γ-terpinene synthase, terpinolene synthase, amorphadiene synthase, α-farnesene synthase, β-farnesene synthase, farnesol synthase, nerolidol synthase, patchouliol synthase, nootkatone synthase, and abietadiene synthase. In some ~~embodiments~~examples, the isoprenoid is selected from the group consisting of a hemiterpene, monoterpene, diterpene, triterpene, tetraterpene, sesquiterpene, and polyterpene. In some ~~embodiments~~examples, the isoprenoid is a C_5-C_{20} isoprenoid. In some ~~embodiments~~examples, the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, isoprene, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpinolene, and valencene.

[0026] In some ~~embodiments~~examples, the genetically modified host cell is a yeast cell. In some embodiments, the yeast is *Saccharomyces cerevisiae*.

[0027] In another ~~aspect~~example, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate; (b) a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase,

acetylating (ADA); (c) a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthase 1 (ACS1), acetyl-CoA synthase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6); (d) a heterologous nucleic acid encoding a phosphoketolase (PK); and (e) a heterologous nucleic acid encoding a phosphoketolase (PTA).

[0028] In another ~~aspect~~example, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate, wherein the one or more enzymes comprise a NADH-using HMG-CoA reductase; (b) a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); and (c) a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthase 1 (ACS1), acetyl-CoA synthase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6).

[0029] In another ~~aspect~~example, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate, wherein the one or more enzymes comprise a NADH-using HMG-CoA reductase; (b) a heterologous nucleic acid encoding an acetylaldehyde dehydrogenase, acetylating (ADA); (c) a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthase 1 (ACS1), acetyl-CoA synthase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6); (d) a heterologous nucleic acid encoding a phosphoketolase (PK); and (e) a heterologous nucleic acid encoding a phosphoketolase (PTA).

[0030] In another ~~aspect~~example, provided herein is genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding one or more enzymes of a mevalonate (MEV) pathway for making isopentenyl pyrophosphate, wherein the one or more enzymes comprise an acetyl-CoA:malonyl-CoA acyltransferase; (b) a heterologous nucleic acid encoding acetylaldehyde dehydrogenase, acetylating (ADA); and (c) a functional disruption of at least one enzyme of the native PDH-bypass selected from the group consisting of acetyl-CoA synthase 1 (ACS1), acetyl-CoA synthase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6).

[0031] In another ~~aspect~~example, provided herein is a genetically modified host cell capable of producing an isoprenoid, the cell comprising: (a) one or more heterologous nucleic acids encoding a plurality of enzymes of a mevalonate (MEV) pathway for making isopentenyl

29. The genetically modified host cell of any one of claims 1 to 28, further comprising a heterologous nucleic acid encoding an enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound.

30. The genetically modified host cell of claim 29, wherein the enzyme that can modify IPP or a polyprenyl to form an isoprenoid compound is selected from the group consisting of carene synthase, geraniol synthase, linalool synthase, limonene synthase, myrcene synthase, ocimene synthase, α-pinene synthase, β-pinene synthase, γ-terpinene synthase, terpinolene synthase, amorphadiene synthase, α-farnesene synthase, β-farnesene synthase, farnesol synthase, nerolidol synthase, patchouliol synthase, nootkatone synthase, and abietadiene synthase.

~~30.~~31. The genetically modified host cell of claim 29, wherein the isoprenoid is selected from the group consisting of a hemiterpene, monoterpene, diterpene, triterpene, tetraterpene, sesquiterpene, and polyterpene.

~~31.~~32. The genetically modified host cell of claim 29, wherein the isoprenoid is a sesquiterpene.

~~32.~~33. The genetically modified host cell of claim 29, wherein the isoprenoid is a C_5-C_{20} isoprenoid.

~~33.~~34. The genetically modified host cell of claim 29, wherein the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, isoprene, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpinolene, and valencene.

~~34.~~35. The genetically modified host cell of any one of claims 1 to 34, wherein the cell is a yeast cell.

~~35.~~36. A method for producing an isoprenoid comprising:

(a) culturing a population of the genetically modified host cell of any one of claims 1 to 35 in a medium with a carbon source under conditions suitable for making said isoprenoid compound; and

(b) recovering said isoprenoid compound from the medium.