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 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. Thus, in a first aspect, the present invention discloses a genetically modified yeast 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 (ADA). In another aspect, the present invention refers to a method for producing an isoprenoid comprising (a) culturing a population of the genetically modified yeast cell of the present invention in a medium with a carbon source under conditions suitable for making said isoprenoid compound; and (b) recovering said isoprenoid compound from the medium. In still another aspect, the present invention refers to a genetically modified yeast cell capable of producing an isoprenoid, the yeast cell comprising (a) a heterologous nucleic acid encoding an acylating acetylaldehyde dehydrogenase (ADA); and optionally 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 a phosphoketolase (PK); and (c) a heterologous nucleic acid encoding a phosphotransacetylase (PTA), and further comprising a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH)-bypass selected from the group consisting of acetyl-CoA synthetase 1 (ACS 1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6), wherein the genetically modified yeast cell produces an increased amount of an isoprenoid compound compared to an yeast cell which is not genetically modified. In some examples disclosed herein, 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 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 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 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 examples, the formation of acetoacetyl-CoA from acetyl-CoA is catalyzed by an acetoacetyl-CoA synthase (AACS;

WHAT IS CLAIMED:

1. A genetically modified yeast 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 (ADA).

2. The genetically modified yeast cell of claim 1, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA.

3. The genetically modified yeast cell of claim 1 or 2, wherein the one or more enzymes of the MEV pathway comprise an acetyl-CoA:malonyl-CoA acyltransferase.

4. The genetically modified yeast cell of any one of claims 1 to 3, wherein the one or more enzymes of the MEV pathway comprise an NADH-using enzyme that converts 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate.

5. The genetically modified yeast cell of any one of claims 1 to 4, wherein the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase.

6. The genetically modified yeast cell of any one of claims 1 to 5, further comprising a heterologous nucleic acid encoding a phosphoketolase (PK).

7. The genetically modified yeast cell of any one of claims 1 to 6, further comprising a heterologous nucleic acid encoding a phosphotransacetylase (PTA).

8. The genetically modified yeast cell of any one of claims 1 to 7, further comprising a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH)-bypass.

9. The genetically modified yeast cell of claim 8, wherein 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).

10. The genetically modified yeast cell of claim 9, wherein ACS1 is functionally disrupted.

11. The genetically modified yeast cell of claim 9, wherein ACS2 is functionally disrupted.

12. The genetically modified yeast cell of claim 9, wherein ALD6 is functionally disrupted.

13. The genetically modified yeast cell of claim 9, wherein ACS1 and ACS2 are functionally disrupted.

14. The genetically modified yeast cell of claim 9, wherein ACS1, ACS2 and ALD6 are functionally disrupted.

15. The genetically modified yeast cell of any one of claims 1 to 14, further comprising a functional disruption of one or more enzymes having alcohol dehydrogenase (ADH) activity.

16. The genetically modified yeast cell of claim 15, wherein 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).

17. The genetically modified yeast cell of any one of claims 1 and 4 to 16, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA.

18. The genetically modified yeast cell of any one of claims 1 to 17, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA.

19. The genetically modified yeast cell of any one of claims 1 to 3 and 6 to 17, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate.

20. The genetically modified yeast cell of any one of claims 1 to 19, wherein the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate.

21. The genetically modified yeast cell of any one of claims 1 to 20, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate.

22. The genetically modified yeast cell of any one of claims 1 to 21, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

23. The genetically modified yeast cell of any one of claims 1 to 22, wherein the one or more enzymes of the MEV pathway are selected from HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase.

24. The genetically modified yeast cell of any one of claims 1 to 23, wherein the yeast cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway.

25. The genetically modified yeast cell of any one of claims 1 to 24, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of a single transcriptional regulator.

26. The genetically modified yeast cell of any one of claims 1 to 24, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of multiple heterologous transcriptional regulators.

27. The genetically modified yeast cell of any one of claims 1 to 26, further comprising a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP).

28. The genetically modified yeast cell of any one of claims 1 to 27, further comprising a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound.

29. The genetically modified yeast 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 yeast 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.

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

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

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

34. The genetically modified yeast 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.

35. The genetically modified yeast cell of any one of claims 1 to 34, wherein the yeast cell is *Saccharomyces cerevisiae*.

36. A method for producing an isoprenoid comprising:

(a) culturing a population of the genetically modified yeast 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.

37. A genetically modified yeast cell capable of producing an isoprenoid, the yeast cell comprising:

(a) a heterologous nucleic acid encoding an acylating acetylaldehyde dehydrogenase (ADA); and optionally 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 a phosphoketolase (PK); and

(c) a heterologous nucleic acid encoding a phosphotransacetylase (PTA), and

further comprising a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH)-bypass selected from the group consisting of acetyl-CoA synthetase 1 (ACS 1), acetyl-CoA synthetase 2 (ACS2), and aldehyde dehydrogenase 6 (ALD6),

wherein the genetically modified yeast cell produces an increased amount of an isoprenoid compound compared to an yeast cell which is not genetically modified.

38. The genetically modified yeast cell of claim 37, further comprising a heterologous nucleic acid encoding an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA.

39. The genetically modified yeast cell of claim 38, wherein the enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA is an acetyl-CoA malonyl-CoA acyltransferase.

40. The genetically modified yeast cell of claim 37, wherein the one or more enzymes of the MEV pathway comprise an NADH-using enzyme that converts 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate.

41. The genetically modified yeast cell of claim 37, wherein the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase (HMGr).

42. The genetically modified yeast cell of any one of claims 37 to 41, wherein ACS1, ACS2, or both are functionally disrupted.

43. The genetically modified yeast cell of any one of claims 37 to 42, wherein ALD6 is functionally disrupted.

44. The genetically modified yeast cell of any one of claims 37 to 43, further comprising the one or more heterologous nucleic acids encoding the one or more enzymes of the MEV pathway selected from the group consisting of an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA, an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA, an NAPDH-using enzyme that converts HMG-CoA to mevalonate, an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate, an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate, and an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

45. The genetically modified yeast cell of any one of claims 37 to 44, further comprising a heterologous nucleic acid encoding an enzyme that converts isopentenyl pyropyosphate (IPP) to dimethylallyl pyrophosphate (DMAPP).

46. The genetically modified yeast cell of claim 45, further comprising a heterologous nucleic acid encoding an enzyme that condenses IPP and/or DMAPP molecules to form a polyprenyl compound and a heterologous nucleic acid encoding an enzyme that modifies IPP or the polyprenyl compound to form an isoprenoid compound.

47. The genetically modified yeast cell of claim 46, wherein the isoprenoid is selected from the group consisting of a hemiterpene, monoterpene, diterpene, triterpene, tetraterpene, sesquiterpene, and polyterpene.

WHAT IS CLAIMED:

1. 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 (ADA).

2. The genetically modified host cell of claim 1, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetyl-CoA with malonyl-CoA to form acetoacetyl-CoA.

3. The genetically modified host cell of claim 1 or claim 2, wherein the one or more enzymes of the MEV pathway comprise an acetyl-CoA:malonyl-CoA acyltransferase.

4. The genetically modified host cell of any one of claims 1 to 3, wherein the one or more enzymes of the MEV pathway comprise an NADH-using enzyme that converts 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate.

5. The genetically modified host cell of any one of claims 1 to 4, wherein the one or more enzymes of the MEV pathway comprise an NADH-using HMG-CoA reductase.

6. The genetically modified host cell of any one of claims 1 to 5, further comprising a heterologous nucleic acid encoding a phosphoketolase (PK).

7. The genetically modified host cell of any one of claims 1 to 6, further comprising a heterologous nucleic acid encoding a phosphotransacetylase (PTA).

8. The genetically modified host cell of any one of claims 1 to 7, further comprising a functional disruption of one or more enzymes of the native pyruvate dehydrogenase (PDH) -bypass.

9. The genetically modified host cell of claim 8, wherein 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).

10. The genetically modified host cell of claim 9, wherein ACS1 is functionally disrupted.

11. The genetically modified host cell of claim 9, wherein ACS2 is functionally disrupted.

12. The genetically modified host cell of claim 9, wherein ALD6 is functionally disrupted.

13. The genetically modified host cell of claim 9, wherein ACS1 and ACS2 are functionally disrupted.

14. The genetically modified host cell of claim 9, wherein ACS1, ACS2 and ALD6 are functionally disrupted.

15. The genetically modified host cell of any one of claims 1 to 14, further comprising a functional disruption of one or more enzymes having alcohol dehydrogenase (ADH) activity.

16. The genetically modified host cell of claim 15, wherein 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).

17. The genetically modified host cell of any one of claims 1 and 4 to 16, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses two molecules of acetyl-CoA to form acetoacetyl-CoA.

18. The genetically modified host cell of any one of claims 1 to 17, wherein the one or more enzymes of the MEV pathway comprise an enzyme that condenses acetoacetyl-CoA with acetyl-CoA to form HMG-CoA.

19. The genetically modified host cell of any one of claims 1 to 3 and 6 to 17, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts HMG-CoA to mevalonate.

20. The genetically modified host cell of any one of claims 1 to 19, wherein the one or more enzymes of the MEV pathway comprise an enzyme that phosphorylates mevalonate to mevalonate 5-phosphate.

21. The genetically modified host cell of any one of claims 1 to 20, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-phosphate to mevalonate 5-pyrophosphate.

22. The genetically modified host cell of any one of claims 1 to 21, wherein the one or more enzymes of the MEV pathway comprise an enzyme that converts mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

23. The genetically modified host cell of any one of claims 1 to 22, wherein the one or more enzymes of the MEV pathway are selected from HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase and mevalonate pyrophosphate decarboxylase.

24. The genetically modified host cell of any one of claims 1 to 23, wherein the host cell comprises a plurality of heterologous nucleic acids encoding all of the enzymes of the MEV pathway.

25. The genetically modified host cell of any one of claims 1 to 24, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of a single transcriptional regulator.

26. The genetically modified host cell of any one of claims 1 to 24, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the MEV pathway are under control of multiple heterologous transcriptional regulators.

27. The genetically modified host cell of any one of claims 1 to 26, further comprising a heterologous nucleic acid encoding an enzyme that can convert isopentenyl pyrophosphate (IPP) into dimethylallyl pyrophosphate (DMAPP).

28. The genetically modified host cell of any one of claims 1 to 27, further comprising a heterologous nucleic acid encoding an enzyme that can condense IPP and/or DMAPP molecules to form a polyprenyl compound.

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.

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.

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

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

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.

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

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.