result, are currently uneconomical or impossible to synthesize. Such natural products must be either extracted from their native sources, such as trees, sponges, corals and marine microbes; or produced synthetically or semi-synthetically from more abundant precursors. Extraction of a natural product from a native source is limited by the availability of the native source; and synthetic or semi-synthetic production of natural products can suffer from low yield and/or high cost. Such production problems and limited availability of the natural source can restrict the commercial and clinical development of such products.

[0005] An example of an important sesquiterpene compound is artemisinin. Artemisinin is a highly effective anti-malarial drug that is currently extracted from plants (*Artemisia annua)* and is used to make combination therapy medications. Plant-derived artemisinin is expensive and its availability is subject to weather and political conditions in the countries that grow the plants. Artemisinic acid is a key intermediate in the biosynthesis of artemisinin. Conversion of amorpha-4,11-diene to artemisinic alcohol, an important step in making artemisinin, by traditional chemistry is a difficult and costly process.

[0006] There is a need in the art for methods of generating isoprenoid compounds that avoid some of the above-mentioned drawbacks. The present invention addresses this need by providing polynucleotides that encode enzymes that modify isoprenoid compounds, and host cells that are genetically modified to produce such enzymes.

Literature

[0007] Bertea et al. (2005) *Planta Med.* 71:40-47; deKraker et al. (2003) *Tetradedron* 59:409-418; Martin et al. (2003) *Nat. Biotechnol.* 21:796-802; WO 03/025193; U.S. Patent Publication No. 20050019882; U.S. Patent Publication No. 20030148479; U.S. Patent Publication No. 20040005678; U.S. Patent Publication No. 20030166255.

SUMMARY ~~OF THE INVENTION~~

[0008] The present ~~invention~~disclosure provides isolated nucleic acids comprising nucleotide sequences encoding isoprenoid modifying enzymes, as well as recombinant vectors comprising the nucleic acids. The present ~~invention~~disclosure further provides genetically modified host cells comprising a subject nucleic acid or recombinant vector. The present ~~invention~~disclosure further provides a transgenic plant comprising a subject nucleic acid. The present ~~invention~~disclosure further provides methods of producing an isoprenoid compound, the method generally involving culturing a subject genetically modified host cell under conditions that permit synthesis of an enzyme encoded by a subject nucleic acid, which enzyme modifies an isoprenoid compound.

[0008a] In a first aspect of the present disclosure, there is provided an isolated cDNA comprising: a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

[0008b] In a second aspect of the present disclosure, there is provided a recombinant vector comprising a polynucleotide comprising a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

[0008c] In a third aspect of the present disclosure, there is provided a host cell comprising the cDNA as defined above.

[0008d] In a fourth aspect of the present disclosure, there is provided a host cell comprising the recombinant vector as defined above.

[0008e] In a fifth aspect of the present disclosure, there is provided a transgenic plant comprising a nucleic acid comprising a nucleotide sequence encoding as isoprenoid-modifying enzyme having at least about 90% amino acid identity to the amino acid sequence set forth in SEQ ID NO:2, wherein the nucleic acid is expressed in a cell of the plant to produce the isoprenoid-modifying enzyme in the cell.

[0008f] In a sixth aspect of the present disclosure, there is provided a method of producing an isoprenoid compound, the method comprising maintaining the transgenic plant as defined above under conditions that favor production of the isoprenoid-modifying enzyme, wherein production of the isoprenoid-modifying enzyme results in modification of a terpene substrate and production of an isoprenoid compound.

[0008g] In a seventh aspect of the present disclosure, there is provided a transgenic plant genetically modified with a nucleic acid comprising a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that encodes an enzyme that modifies an isoprenoid compound, wherein the nucleotide sequence has at least 60% nucleotide sequence identity with the nucleotide sequence set forth in SEQ ID NO:1.

2. The polynucleotide of claim 1, wherein the wherein the nucleotide sequence has at least 80% nucleotide sequence identity with the nucleotide sequence set forth in SEQ ID NO:1.

3. The polynucleotide of claim 1, wherein the wherein the nucleotide sequence has at least 90% nucleotide sequence identity with the nucleotide sequence set forth in SEQ ID NO:1.

4. The polynucleotide of claim 1, wherein the polynucleotide comprises a nucleotide sequence that encodes a polypeptide having at least about 45% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

5. The polynucleotide of claim 4, wherein the nucleotide sequence encodes a polypeptide having at least about 65% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

6. The polynucleotide of claim 4, wherein the nucleotide sequence encodes a polypeptide having at least about 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

7. A recombinant vector comprising the polynucleotide of claim 1.

8. A host cell comprising the polynucleotide of claim 1.

9. A host cell comprising the recombinant vector of claim 7.

10. A method of producing an isoprenoid compound in a host cell, the method comprising:

culturing a genetically modified host cell in a suitable medium, wherein said host cell is genetically modified with a nucleic acid comprising a nucleotide sequence encoding an isoprenoid-modifying enzyme having at least about 45% amino acid identity to the amino acid sequence set forth in SEQ ID NO:2, to produce an isoprenoid-modifying enzyme,

wherein, in the presence of a terpene substrate, said production of said isoprenoid-modifying enzyme results in enzymatic modification of the terpene substrate and production of the isoprenoid compound.

11. The method of claim 10, wherein said isoprenoid-modifying enzyme is an amorphadiene oxidase, and wherein the terpene substrate is amorpha-4,11-diene.

12. The method of claim 10, wherein said host cell is a eukaryotic host cell.

13. The method of claim 12, wherein said host cell is a yeast cell.

14. The method of claim 12, wherein said host cell is a plant cell.

15. The method of claim 10, wherein said host cell is further genetically modified with a nucleic acid comprising a nucleotide sequence encoding a terpene synthase, wherein said culturing provides for production of said terpene synthase, wherein said terpene synthase modifies a farnesyl pyrophosphate to generate a sesquiterpene substrate for said isoprenoid-modifying enzyme.

16. The method of claim 10, wherein said host cell is further genetically modified with a nucleic acid comprising a nucleotide sequence encoding a terpene synthase, wherein said culturing provides for production of said terpene synthase, wherein said terpene synthase modifies a geranyl pyrophosphate to generate a monoterpene substrate for said isoprenoid-modifying enzyme.

17. The method of claim 10, wherein said host cell is further genetically modified with a nucleic acid comprising a nucleotide sequence encoding a terpene synthase, wherein said culturing provides for production of said terpene synthase, wherein said terpene

synthase modifies a geranylgeranyl pyrophosphate to generate a diterpene substrate for said isoprenoid-modifying enzyme.

18. The method of claim 10, wherein said host cell is one that does not normally synthesize isopentenyl pyrophosphate (IPP) via a mevalonate pathway, and wherein the host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding two or more enzymes in the mevalonate pathway, an IPP isomerase, a prenyltransferase, and a terpene synthase, said culturing providing for production of the mevalonate pathway enzymes, wherein said production of said two or more mevalonate pathway enzymes, said IPP isomerase, said prenyltransferase, said terpene synthase, and said isoprenoid-modifying enzyme results in production of an isoprenoid compound.

19. The method of claim 18, wherein said two or more mevalonate pathway enzymes comprises mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase, and wherein the host cell is cultured in the presence of mevalonate.

20. The method of claim 18, wherein said two or more mevalonate pathway enzymes comprises acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

21. The method of claim 18, wherein said prenyltransferase is a farnesyl pyrophosphate synthase.

22. The method of claim 10, wherein said sesquiterpene comprises an isopropenyl group.

23. The method of claim 10, wherein said isoprenoid-modifying enzyme catalyzes oxidation of an isopropenyl group of the terpene substrate.

24. The method of claim 10, wherein said terpene substrate is a sesquiterpene.

25. The method of claim 10, wherein said isoprenoid-modifying enzyme catalyzes the C12 oxidation of amorphadiene.

26. The method of claim 24, wherein said sesquiterpene is selected from amorphadiene, alloisolongifolene, (-)-α-*trans*-bergamotene, (-)-β-elemene, (+)-germacrene A, germacrene B, (+)-γ-gurjunene, (+)-ledene, neointermedeol, (+)-β-selinene, and (+)-valencene.

27. The method of claim 10, wherein said terpene substrate is a monoterpene.

28. The method of claim 10, wherein said terpene substrate is a diterpene.

29. The method of claim 10, wherein said terpene substrate is a triterpene.

30. The method of claim 10, wherein said terpene substrate is a polyterpene.

31. The method of claim 10, wherein said isoprenoid-modifying enzyme-encoding nucleotide sequence has at least about 60% nucleotide sequence identity to the nucleotide sequence set forth in SEQ ID NO:1.

32. The method of claim 10, wherein said host cell is genetically modified with a nucleic acid comprising a nucleotide sequence encoding a cytochrome P450 reductase (CPR).

33. The method of claim 10, wherein said isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to an inducible promoter.

34. The method of claim 10, wherein the host cell is a prokaryotic host cell.

35. The method of claim 31, wherein the prokaryotic host cell is *Escherichia coli*.

36. The method of claim 34, wherein the prokaryotic host cell is one that normally synthesizes IPP through the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway.

37. The method of claim 36, wherein the DXP pathway is inactivated.

38. The method of claim 10, further comprising isolating the isoprenoid compound.

39. The method of claim 11, wherein the isoprenoid compound is artemisinic acid.

40. The method of claim 39, further comprising modifying artemisinic acid to generate artemisinin.

41. An isolated polynucleotide comprising a nucleotide sequence that encodes a cytochrome P450 reductase, wherein the nucleotide sequence has at least 90% nucleotide sequence identity with the nucleotide sequence set forth in SEQ ID NO:3.

42. A recombinant vector comprising the polynucleotide of claim 41.

43. A host cell comprising the polynucleotide of claim 41.

44. A host cell comprising the recombinant vector of claim 42.

45. A method of hydroxylating a terpene compound, the method comprising culturing a genetically modified host cell in a suitable medium, wherein said host cell is genetically modified with a nucleic acid comprising a nucleotide sequence encoding an isoprenoid-modifying enzyme having at least about 45% amino acid identity to the amino acid sequence set forth in SEQ ID NO:2, to produce an isoprenoid-modifying enzyme, wherein, in the presence of a terpene compound, said production of said isoprenoid-modifying enzyme results in hydroxylation of the terpene compound.

46. A transgenic plant comprising a nucleic acid comprising a nucleotide sequence encoding as isoprenoid -modifying enzyme having at least about 45% amino acid identity to the amino acid sequence set forth in SEQ ID NO:2, wherein the nucleic acid is expressed in a cell of the plant to produce the isoprenoid-modifying enzyme in the cell.

47. The transgenic plant of claim 46, wherein the plant is a monocot.

48. The transgenic plant of claim 46, wherein the plant is a dicot.

49. The transgenic plant of claim 46, wherein the plant is tobacco.

50. The transgenic plant of claim 46, wherein the isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to a constitutive promoter.

51. The transgenic plant of claim 46, wherein the isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to an inducible promoter.

52. The transgenic plant of claim 46, wherein the isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to a tissue-specific promoter.

53. The transgenic plant of claim 52, wherein the tissue-specific promoter is a trichome-specific promoter.

54. The transgenic plant of claim 46, wherein the plant is *Artemisia annua*.

55. A method of producing an isoprenoid compound, the method comprising maintaining the transgenic plant of claim 46 under conditions that favor production of the isoprenoid-modifying enzyme, wherein production of the isoprenoid-modifying enzyme results in modification of a terpene substrate and production of an isoprenoid compound.

CLAIMS

What is claimed is:

1. An isolated cDNA comprising:

a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

2. A recombinant vector comprising a polynucleotide comprising a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

3. A host cell comprising the cDNA of claim 1.

4. A host cell comprising the recombinant vector of claim 2.

5. A transgenic plant comprising a nucleic acid comprising a nucleotide sequence encoding as isoprenoid-modifying enzyme having at least about 90% amino acid identity to the amino acid sequence set forth in SEQ ID NO:2, wherein the nucleic acid is expressed in a cell of the plant to produce the isoprenoid-modifying enzyme in the cell.

6. The transgenic plant of claim 5, wherein the plant is a monocot.

7. The transgenic plant of claim 5, wherein the plant is a dicot.

8. The transgenic plant of claim 5, wherein the plant is tobacco.

9. The transgenic plant of claim 5, wherein the isoprenoid-modifying enzyme-encoding

nucleotide sequence is operably linked to a constitutive promoter.

10. The transgenic plant of claim 5, wherein the isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to an inducible promoter.

11. The transgenic plant of claim 5, wherein the isoprenoid-modifying enzyme-encoding nucleotide sequence is operably linked to a tissue-specific promoter.

12. The transgenic plant of claim 11, wherein the tissue-specific promoter is a trichome-specific promoter.

13. The transgenic plant of claim 5, wherein the plant is *Artemisia annua*.

14. A method of producing an isoprenoid compound, the method comprising maintaining the transgenic plant of claim 5 under conditions that favor production of the isoprenoid-modifying enzyme, wherein production of the isoprenoid-modifying enzyme results in modification of a terpene substrate and production of an isoprenoid compound.

15. The cDNA of claim 1, wherein the CPR comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4.

16. The cDNA of claim 1, wherein the CPR comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4.

17. The recombinant vector of claim 2, wherein said nucleotide sequence is operably linked to a promoter.

18. The recombinant vector of claim 2, further comprising a nucleic acid encoding an isoprenoid-modifying enzyme having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

19. The recombinant vector of claim 2, wherein the isoprenoid-modifying enzyme exhibits amorpha-4,11-diene oxidase activity.

20. The host cell of claim 4, wherein the host further comprises an expression vector encoding an amorpha-4,11-diene oxidase having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2.

21. The host cell of claim 3, wherein the host cell is a prokaryotic cell, a yeast cell, or a plant cell.

22. The host cell of claim 4, wherein the host cell is a prokaryotic cell, a yeast cell, or a plant cell.

23. A transgenic plant genetically modified with a nucleic acid comprising a nucleotide sequence that encodes a cytochrome P450 reductase (CPR), wherein the CPR comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4, wherein the CPR transfers electrons from NADPH to an isoprenoid-modifying enzyme.

24. A transgenic plant according to claim 23, further comprising a polypeptide having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, wherein the polypeptide exhibits amorpha-4,11-diene oxidase activity.

25. The transgenic plant of claim 23, wherein the plant is tobacco or *Artemisia annua*.

26. The recombinant vector of claim 2, wherein the CPR comprises an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 4.

27. The recombinant vector of claim 2, wherein the CPR comprises an amino acid sequence having at least 95% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 4.