viridiflorol. Chemical synthesis of viridiflorol has low enantioselectivity. The existence of 6 chiral centres, or 64 different stereoisomers, has posed a great challenge to the chemical synthesis of viridiflorol, resulting in low yields and ultra-high costs.

[0005] There is therefore a need to identify methods of viridiflorol synthesis that can produce higher yields.

SUMMARY

[0006] In one aspect, there is provided a method of increasing terpenoid production in a host cell that produces one or more terpenoids, comprising:

a) providing a host cell that produces one or more terpenoids, said host cell comprising a vector comprising a polynucleotide sequence encoding a terpene synthase enzyme;

b) modifying the vector to:

i. introduce an inducible promoter operably linked to the polynucleotide sequence encoding the terpene synthase enzyme; and

ii. introduce a polynucleotide sequence encoding a ribosomal binding site (RBS) that increases translation initiation rate of the terpenoid compared to a wild type ribosomal binding site;

c) determining the dosage of an inducer capable of inducing the inducible promoter;

d) culturing the host cell in a culture medium in the presence of the inducer at the dosage determined from step c); and

e) isolating the terpenoid from the culture medium.

[0006a] In another aspect, provided herein is a method of increasing viridiflorol production in a host cell that produces one or more terpenoids, comprising:

a) providing a host cell that produces one or more terpenoids, said host cell comprising a vector comprising a polynucleotide sequence encoding a terpene synthase enzyme, wherein the terpene synthase enzyme is viridiflorol synthase isolated from *Agrocybe aegerita*;

b) modifying the vector to:

i. introduce an inducible promoter operably linked to the polynucleotide sequence encoding the terpene synthase enzyme; and

ii. introduce a polynucleotide sequence encoding a ribosomal binding site (RBS) that increases translation initiation rate of the terpene synthase enzyme compared to a wild type ribosomal binding site;

c) determining the dosage of an inducer capable of inducing the inducible promoter;

d) culturing the host cell in a culture medium in the presence of the inducer at the dosage determined from step c).

[0007] In another aspect, there is provided a host cell comprising at least one vector, wherein said at least one vector comprises:

a) a polynucleotide sequence encoding one or more genes of the mevalonate pathway operably linked to an inducible promoter;

b) a polynucleotide sequence encoding a *ispA* gene;

c) a polynucleotide sequence encoding a terpene synthase enzyme operably linked to an inducible promoter; and

d) a polynucleotide sequence encoding a ribosomal binding site (RBS) that increases translation initiation rate of the terpene synthase enzyme compared to a wild type ribosomal binding site.

Claims

1. A method of increasing viridiflorol production in a host cell that produces one or more terpenoids, comprising:
   a) providing a host cell that produces one or more terpenoids, said host cell comprising a vector comprising a polynucleotide sequence encoding a terpene synthase enzyme, wherein the terpene synthase enzyme is viridiflorol synthase isolated from *Agrocybe aegerita*;
   b) modifying the vector to:
      i. introduce an inducible promoter operably linked to the polynucleotide sequence encoding the terpene synthase enzyme; and
      ii. introduce a polynucleotide sequence encoding a ribosomal binding site (RBS) that increases translation initiation rate of the terpene synthase enzyme compared to a wild type ribosomal binding site;
   c) determining the dosage of an inducer capable of inducing the inducible promoter;
   d) culturing the host cell in a culture medium in the presence of the inducer at the dosage determined from step c).

2. The method of claim 1, wherein the host cell comprises one or more additional vectors, said one or more additional vectors comprising a polynucleotide sequence encoding one or more genes of the mevalonate pathway operably linked to an inducible promoter.

3. The method of claim 2, wherein the host cell further comprises two additional vectors, wherein the first additional vector comprises a polynucleotide sequence encoding the genes *atoB, hmgS* and truncated *hmgR* of the mevalonate pathway and the second additional vector comprises a polynucleotide sequence encoding the genes *mevK*, *pmK*, *pmd* and *idi* of the mevalonate pathway, wherein the genes of the mevalonate pathway are from *Saccharomyces cerevisiae* and/or *Escherichia coli.*

4. The method of any one of claims 1-3, wherein the inducible promoter is a T7 promoter variant selected from the group consisting of TM1, TM2 and TM3, wherein TM1, TM2 and TM3 have the polynucleotide sequence of SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30 respectively.

5. The method of claim 4, wherein the inducible promoter in the vector comprising a polynucleotide sequence encoding a terpene synthase enzyme is TM1, the inducible promoter in the first additional vector comprising the *atoB, hmgS* and truncated *hmgR* genes is TM2, and the inducible promoter in the second additional vector comprising the *mevK*, *pmK*, *pmd* and *idi* genes is TM3.

6. The method of any one of claims 1-5, wherein the polynucleotide sequence encoding the RBS is situated upstream of the polynucleotide sequence encoding the terpene synthase enzyme.

7. The method of claim 6, wherein the polynucleotide sequence encoding the RBS is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 17.

8. The method of any one of claims 1-7, wherein the inducer is lactose or isopropyl β-D-1-thiogalactopyranoside (IPTG).

9. The method of any one of claims 1-8, wherein the inducer dosage is determined using the product of the total promoter strength and the strength of the promoter operably linked to the *mevK*, *pmK*, *pmd* and *idi* genes.

10. The method of any one of claims 1-9, wherein the inducer dosage is between 0.001-0.5 mM IPTG or between 0.1-50 mM lactose.

11. The method of claim 10, wherein the host cell is an *Escherichia coli* cell.

12. The method of claim 11, wherein the *Escherichia coli* is a DE3 strain encoding a T7 RNA polymerase integrated into its genome.

13. The method of any one of claims 1-12, wherein the host cell is deficient in at least one gene involved in amino acid synthesis.

14. The method of claim 13, wherein the vector comprising a polynucleotide sequence encoding a terpene synthase enzyme and the one or more additional vectors further comprises a polynucleotide sequence encoding the one or more genes that the host cell is deficient in.

15. The method of claims 13 or 14, wherein the at least one gene is selected from the group consisting of *aroA, aroB and aroC*, and wherein the at least one gene is from *Escherichia coli.*

16. The method of any one of claims 1-15, wherein the vector comprising a polynucleotide sequence encoding a terpene synthase enzyme further comprises a

polynucleotide sequence encoding the gene *aroA*.

17. The method of any one of claims 3-16, wherein the first additional vector comprising a polynucleotide sequence encoding the genes *atoB, hmgS* and truncated *hmgR* further comprises a polynucleotide sequence encoding the gene *aroC,* and wherein the second additional vector comprises a polynucleotide sequence encoding the genes *mevK*, *pmK*, *pmd* and *idi* further comprises a polynucleotide sequence encoding the gene *aroB*.

18. The method of claim 1, wherein the viridiflorol synthase is mutated at one or more amino acid positions.

19. The method of claim 1, wherein the mutation is G227C, V314Y and deletion of amino acids at positions 2 to 85 of SEQ ID NO: 24.

20. The method of any one of claims 1-19, wherein viridiflorol production is increased by at least 2000 fold compared to a host cell that produces one or more terpenoids comprising a vector comprising a polynucleotide sequence encoding a terpene synthase enzyme that has not been modified.