BIOSYNTHESIS OF LINALOOL

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore application No. 10202201742V, filed 22 February 2022, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates to the biosynthesis of linalool.

BACKGROUND OF THE INVENTION

[0003] Linalool is a floral monoterpene alcohol and natural linalool can be found in many flowers (e.g., lavender and rose) or citrus fruits. Natural linalool exists in two stereoisomers, namely (S)-linalool and (R)-linalool, each bearing a unique smell and taste. Importantly, linalool is a commercially important fragrance molecule that is widely used in food, beverage, cosmetics, and personal care products (e.g., perfumes, detergents, shampoos, and lotions etc.). Linalool also serves as the starting material to the manufacturing of vitamin E.

[0004] The yield of linalool extracted from natural plant tissues is low. Thus, the current supply of linalool is predominantly produced by chemical synthesis. However, this method produces racemic linalool, which is unsustainable, environmentally unfriendly, and less favoured by consumers. Various researchers are exploring the biotechnological production of linalool, but the great majority have achieved limited success. Moreover, biosynthesis of linalool can only produce enantiopure linalool, either (S)-linalool and (R)-linalool. Therefore, there is a need to provide a novel green and cost-effective method of synthesizing linalool. that overcomes, or at least ameliorates, one or more of the disadvantages described above.

SUMMARY

[0005] In one aspect, provided herein is a host cell comprising one or more vectors comprising a polynucleotide sequence encoding: a) mevalonate pathway genes; and b) linalool pathway genes, wherein the linalool pathway genes comprise more than one diphosphate synthase gene, prenyltransferase gene or combinations thereof that encode a

protein comprising geranyl pyrophosphate synthase (GPPS) activity, and at least one linalool synthase gene, wherein the host cell is an engineered bacterial cell.

Claims

1. A host cell comprising one or more vectors comprising a polynucleotide sequence encoding:
   a) mevalonate pathway genes; and
   b) linalool pathway genes,

   wherein the linalool pathway genes comprise more than one diphosphate synthase gene, prenyltransferase gene or combinations thereof that encode a protein comprising geranyl pyrophosphate synthase (GPPS) activity, and at least one linalool synthase gene, and wherein the host cell is an engineered bacterial cell.

2. The host cell according to claim 1, wherein
   a) the mevalonate pathway genes are encoded by one or more polynucleotide sequences on one or more vectors; and
   b) the linalool pathway genes are encoded by one or more polynucleotide sequences on one or more vectors, or wherein the linalool pathway genes are inserted into the genome of the host cell.

3. The host cell according to claim 1 or 2, wherein the mevalonate pathway genes are selected from the group consisting of *atoB*, *hmgS, hmgR*, *mevK*, *pmK*, *pmd* and *idi,* and the more than one diphosphate synthase gene, prenyltransferase gene or combinations thereof is a geranyl pyrophosphate synthase (GPPS) gene.

4. The host cell according to claim 3, wherein the *hmgR* gene is truncated, wherein the truncated *hmgR* comprises the polynucleotide sequence as set forth in SEQ ID NO: 7.

5. The host cell according to any one of claims 1 to 4, wherein the linalool synthase gene is isolated from a eukaryote.

6. The host cell according to claim 5, wherein the eukaryote is a fungus selected from the group consisting of *Agrocybe pediades*, *Galerina marginate*, *Hypholoma sublateritium*, *Hebeloma cylindrosporum, Lactarius deliciosus*, *Psilocybe cyanescens*, *Hypholoma fasciculare, Dictyostelium discoideum, Aspergillus calidoustus, Puccinia graminis f. sp. tritici, Melampsora larici-populina, Physarum polycephalum, Paxillus rubicundulus, and Postia placenta.*

7. The host cell according to claim 6, wherein the linalool synthase gene is isolated from *Agrocybe pediades* (ApLS).

8. The host cell according to claim 7, wherein the ApLS is truncated at the C-terminal, optionally wherein the ApLS comprises the polypeptide sequence as set forth in SEQ ID NO. 11.
9. The host cell according to any one of claims 3 to 8, wherein the more than one GPPS gene is isolated from a prokaryote, a eukaryote, a plant, or combinations thereof.
10. The host cell according to claim 9, wherein at least one of the GPPS gene is isolated from *Escherichia coli.*
11. The host cell according to claim 10, wherein the GPPS gene isolated from *Escherichia coli* is a farnesyl pyrophosphate synthase (FPPS) mutated at one amino acid position, wherein the mutated FPPS is $IspA^{S80F}$ comprising the polypeptide sequence as set forth in SEQ ID NO. 12.
12. The host cell according to claim 9, wherein at least one of the GPPS gene is isolated from *Abies grandis* (AgGPPS).
13. The host cell according to claim 12, wherein the GPPS isolated from *Abies grandis* (AgGPPS) is truncated at the N-terminal.
14. The host cell according to claim 13, wherein the truncated AgGPPS comprises the polypeptide sequence as set forth in SEQ ID NO.: 13.
15. The host cell according to any one of the claims 1 to 14, wherein the polynucleotide sequence in each of the vectors is operably linked to one or more inducible promoters, wherein the one or more inducible promoter is a wild-type T7 RNA polymerase promoter or a variant of the wild-type T7 RNA polymerase promoter, wherein the variant of the wild-type T7 RNA polymerase promoter is selected from the group consisting of TM1, TM2, TM3 and combinations thereof.
16. The host cell according to any one of claims 3 to 15, wherein the more than one GPPS is titrated by ribosomal binding site (RBS) engineering.
17. The host cell according to claim 16, wherein the RBS comprises the polynucleotide sequence as set forth in SEQ ID NO: 23 or SEQ ID NO: 24.
18. The host cell according to any one of claims 1 to 17, wherein the host cell is deficient in at least one gene involved in amino acid degradation, wherein the at least one gene involved in amino acid degradation is *tnaA*.
19. The host cell according to claim 1, wherein the bacterial cell is an *Escherichia coli* cell.

20. A method of producing linalool comprising culturing the host cell according to claims 1 to 19 in a culture medium, wherein the culture medium optionally comprises an inducer and at least one carbon substrate.
21. The method according to claim 20, wherein the inducer is lactose or IPTG, and the at least one carbon substrate is selected from the group consisting of acetate, glycerol, glucose and lactose, and combinations thereof.
22. The method according to claims 20 or 21, wherein the titre of linalool production is at least 3000 mg/L.
23. The method according to any one of claims 20 to 22, wherein the yield of linalool production is determined by the concentration of linalool per OD_{600}, and wherein the yield of linalool production is at least 240 mg/L/OD_{600}.
24. The method according to any one of claims 20 to 23, wherein the carbon yield of linalool production is at least 15% of the total carbon mass.
25. A kit for producing linalool, wherein the kit comprises the host cell of any one of claims 1 to 19 with instructions for use.
26. The kit according to claim 25, wherein the host cell is dissolved in solution or lyophilized.
27. The kit according to claim 26, wherein the host cell is preserved by deep freezing.