effective medicines for skin diseases, the retinol market size is estimated to be about 1.6 billion dollars.

[0005] Despite their high commercial values, supply of natural ionones and retinoids is severely limited by their extremely low abundance in nature. Ionones are present in sub-ppm level in many flowers and fruits, such as rose, sweet osmanthus, orris root and raspberry. For instance, it requires 100 tons of raspberries, or 20 hectares of agricultural area, to produce merely 1 gram of α-ionone. As for retinoids, there is no natural source from plants. Exceptionally low amount of retinoids exists in some animal-derived food, such as eggs and butter. Hence, the current supply through extraction from natural sources cannot meet the increasing demands for natural ionones and retinoids. Although these compounds could be chemically synthesized, apocarotenoids such as α-ionone have chiral centres, and synthetic ones are usually a mixture of different enantiomers. The different isomers of many fragrance compounds are known to have different odors, thus it is important to synthesize single isomer instead of a mixture of isomers. More importantly, consumers tend to prefer natural to synthetic flavours and thus natural ingredients have significantly higher prices. The production of α-ionone was previously demonstrated in engineered *Escherichia coli*, but with a very low yield.

[0006] Thus, there is a need to provide naturally produced compounds with improved yields.

SUMMARY

[0007] In one example, there is provided a method for producing a carotenoid or apocarotenoid comprising the step of expressing in a host cell an expression module comprising an expression vector having a coding region encoding at least one optimised carotenoid or apocarotenoid generating enzyme, the coding region being operably linked to a promoter.

[0007a] In one aspect, there is provided a method for producing an apocarotenoid comprising the step of expressing in a host cell an expression module comprising an expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter

[0007b] In another aspect, there is provided a host cell comprising an expression module comprising expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter.

[0007c] In one aspect, there is provided a vector encoding one or more optimised gene products selected from *CCD1*and *LcyE* operably linked to a promoter.

[0007d] In another aspect, there is provided a system for producing a apocarotenoid comprising an expression module comprising an expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter, wherein said at least one optimised apocarotenoid generating enzyme is selected from *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1 for the production of α-ionone.

[0007e] In one aspect, there is provided a kit when used in the method as described herein, for the production of an apocarotenoid comprising one or more of:

a first vector encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY* operably linked to a promoter;

a second vector encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi* operably linked to a promoter;

a third vector encoding one or more optimised third gene products selected from *ispA, crtE, crtB* or *crtI* operably linked to a promoter; and

a fourth vector encoding one or more optimised gene products selected from *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1 operably linked to a promoter for the production of α-ionone.

[0007f] In one aspect, there is provided a method for producing an apocarotenoid comprising the steps of:

a. contacting a host cell as claimed in any one of claims 19 to 24 in a chemically defined media with a substrate for apocarotenoid production;

b. incubating the host cell in said chemically defined media to produce one or more preselected apocarotenoids, and

c. extracting the one or more preselected apocarotenoids from the chemically defined media using an organic layer.

[0008] In one example, there is provided a host cell comprising an expression vector having a coding region encoding at least one optimised carotenoid or apocarotenoid generating enzyme, the coding region being operably linked to a promoter.

[0009] In one example, there is provided a kit when used in the method as described herein, for the production of a carotenoid comprising one or more of: a first vector encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR*; a second vector encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi*

operably linked to a promoter; a third vector encoding one or more optimised third gene products selected from *ispA, crtE or crtB,* optionally *crtI*, operably linked to a promoter for the production of phytoene or lycopene.

[0010] In one example, there is provided a system for producing a carotenoid or apocarotenoid comprising an expression vector having a coding region encoding at least one optimised carotenoid or apocarotenoids generating enzyme, the coding region being operably linked to a promoter, wherein said at least one optimised carotenoid or apocarotenoids generating enzyme is selected from: a. *ΔN50-LsLcyE* and TrxA-CCD1 (preferably TrxA-*Osmanthus fragans* CCD1) for the production of α-ionone; or b. *crtY* and CCD1 (preferably *Petunia hybrid* CCD1) for the production of β-ionone; or c. *ΔN50-LsLcyE* for the production of ε-carotene; or d. *crtY* and *blh* for the production of retinal or retinol.

[0011] In one example, there is provided a kit when used in the method as described herein, for the production of a carotenoid or apocarotenoid comprising one or more of: a first vector encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY* operably linked to a promoter; a second vector encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi* operably linked to a promoter; a third vector encoding one or more optimised third gene products selected from *ispA, crtE, crtB* or *crtI* operably linked to a promoter; and a fourth vector encoding one or more optimised gene products selected from: a. *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1 operably linked to a promoter for the production of α-ionone; or b. *crtY* and *phCCD1* operably linked to a promoter for the production of β-ionone; or c. *ΔN50-LsLcyE* operably linked to a promoter for the production of ε-carotene; or d. *crtY* and *blh* operably linked to a promoter for the production of retinal or retinol.

[0012] In one example, a method for producing a carotenoid or apocarotenoid comprising the steps of: a. contacting a host cell as described herein in a chemically defined media with a substrate for apocarotenoid or carotenoid production; b. incubating the host cell in said chemically defined media to produce one or more preselected carotenoid or apocarotenoid, and c. extracting the one or more preselected carotenoid or apocarotenoids from the chemically defined media using an organic layer.

Claims

1. A method for producing an apocarotenoid comprising the step of expressing in a host cell an expression module comprising an expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter.
2. The method as claimed in claim 1, wherein the apocarotenoid is α-ionone.
3. The method as claimed in claim 1 or 2, wherein said at least one optimised apocarotenoid generating enzyme is selected from *CCD1* and *LcyE*.
4. The method as claimed in claim 1, wherein the apocarotenoid is α-ionone and wherein said at least one optimised apocarotenoid generating enzyme is selected from *LcyE* and *CCD1***,** and form an operon having the structure: *LcyE-CCD1*.
5. The method as claimed in claim 4, wherein said *LcyE* is derived from *Lactuca sativa* and is N-terminal truncated (ranging from 1 to 100 amino acids of *LsLcyE,* especially ΔN50-LsLcyE).
6. The method as claimed in claim 4, wherein said *CCD1* is expressed as a fusion protein selected from *TrxA-CCD1, SUMO-CCD1* or *MBP-CCD1*.
7. The method as claimed in claim 6, wherein the fusion protein is *TrxA-CCD1*.
8. The method as claimed in claim 7, wherein fusion protein is selected from TrxA-*Osmanthus fragrans* CCD1 or TrxA-*Petunia hybrid* CCD1.
9. The method as claimed in claim 8, wherein the CCD1 is derived from or *Osmanthus fragrans* (OfCCD1) and comprise one or more of the following mutations: N154Y, M152T, L151F.
10. The method as claimed in any one of claims 4 to 9, comprising screening for an expression level of α-ionone in an amount of up 100-1000 mg/L in a 24 hour period.
11. The method of any one of claims 1 to 10, further comprising expressing in said host cell: a first expression module comprising an expression vector having a first coding region encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY*, the first coding region being operably linked to a promoter; a second expression module comprising an expression vector having second coding region encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi*, the second coding region being operably linked to a promoter; a third expression module comprising an expression vector having a third coding region encoding one or

more optimised third gene products selected from *ispA, crtE, crtB* or *crtI*, the third coding region being operably linked to a promoter.

12. The method as claimed in claim 11, wherein said one or more first gene products form an operon having the structure: *atoB-hmgS-thmgR.*

13. The method as claimed in claim 11, wherein said one or more second gene products form an operon having the structure: *mevK-pmk-pmd-idi.*

14. The method as claimed in claim 11, wherein said one or more third gene products form an operon having the structure: *crtE-crtB-ispA.*

15. The method as claimed in claim 11, wherein said one or more third gene products form an operon having the structure: *crtE-crtB-crtI-ispA.*

16. The method of any of claims 1 to 15, wherein the host cell is *Escherichia coli* selected from BL21 DE3 or MG1655 DE3.

17. The method as claimed in any one of claims 1 to 16, wherein the promoter is selected from one or more of TM1, TM2 or TM3, T7 RNA polymerase promoter, a T5 RNA polymerase promoter, a T3 RNA polymerase promoter, an SP6 RNA polymerase promoter or an inducible promoter.

18. The method as claimed in any one of claims 1 to 17, wherein the optimisation of the apocarotenoid generating enzymes and optimised gene products is achieved by codon optimisation or site-directed mutagenesis.

19. A host cell comprising an expression module comprising expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter.

20. The host cell as claimed in claim 19, wherein said at least one optimised apocarotenoid generating enzyme is selected from *CCD1*and *LcyE.*

21. The host cell as claimed in claim 20, wherein the optimised gene products are *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1.

22. The host cell as claimed in claim 21, wherein the *Osmanthus fragrans* CCD1 comprises one or more of the following mutations: N154Y, M152T, L151F.

23. The host cell as claimed in any one of claims 11 to 22, further comprising:

a first expression module comprising an expression vector having a first coding region encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY*, the first coding region being operably linked to a promoter;

a second expression module comprising an expression vector having a second coding region encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi*, the second coding region being operably linked to a promoter;

a third expression module comprising an expression vector having a third coding region encoding one or more optimised third gene products selected from *ispA, crtE, crtB* or *crtI*, the third coding region being operably linked to a promoter.

24. The host cell as claimed in any one of claims 11 to 23, wherein the host cell is *Escherichia coli* selected from BL21 DE3 or MG1655 DE3.

25. A vector encoding one or more optimised gene products selected from *CCD1*and *LcyE* operably linked to a promoter.

26. The vector as claimed in claim 25, wherein the optimised gene products are *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1.

27. The vector as claimed in claim 26, wherein the *Osmanthus fragrans* CCD1 comprises one or more of the following mutations: N154Y, M152T, L151F.

28. A system for producing a apocarotenoid comprising an expression module comprising an expression vector having a coding region encoding at least one optimised apocarotenoid generating enzyme, the coding region being operably linked to a promoter, wherein said at least one optimised apocarotenoid generating enzyme is selected from *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1 for the production of α-ionone.

29. The system as claimed in claim 28, further comprising:

a first expression module comprising an expression vector having a first coding region encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY*, the first coding region being operably linked to a promoter;

a second expression module comprising an expression vector having second coding region encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi*, the second coding region being operably linked to a promoter;

a third expression module comprising an expression vector having a third coding region encoding one or more optimised third gene products selected from *ispA, crtE, crtB* or *crtI*, the third coding region being operably linked to a promoter.

30. A kit when used in the method of any of claims 1 to 18, for the production of an apocarotenoid comprising one or more of:

a first vector encoding one or more optimised first gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY* operably linked to a promoter;

a second vector encoding one or more optimised second gene products selected from *mevK, pmk, pmd* or *idi* operably linked to a promoter;

a third vector encoding one or more optimised third gene products selected from *ispA, crtE, crtB* or *crtI* operably linked to a promoter; and

a fourth vector encoding one or more optimised gene products selected from *ΔN50-LsLcyE* and TrxA-*Osmanthus fragrans* CCD1 operably linked to a promoter for the production of α-ionone.

31. A method for producing an apocarotenoid comprising the steps of:

a. contacting a host cell as claimed in any one of claims 19 to 24 in a chemically defined media with a substrate for apocarotenoid production;

b. incubating the host cell in said chemically defined media to produce one or more preselected apocarotenoids, and

c. extracting the one or more preselected apocarotenoids from the chemically defined media using an organic layer.

32. The method as claimed in claim 31, wherein the organic layer is coconut oil or soybean oil.

33. The method as claimed in claim 31 or 32, wherein the apocarotenoid is α-ionone.