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, wherein the apocarotenoid is retinal, or retinol, or a combination of retinal and retinol.

[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.

[0008a] In one 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, wherein said at least one optimised apocarotenoid generating enzyme is selected from *crtY, blh*, *ybbO*, or a combination thereof.

[0009] In one aspect, 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.

[0009a] In one aspect, there is provided a vector encoding one or more optimised gene products selected from *atoB*, *hmgS*, *thmgR* and optionally *crtY* operably linked to a promoter.

[0009b] In one aspect, there is provided a vector encoding one or more optimised gene products selected from *mevK, pmk, pmd* or *idi* operably linked to a promoter.

[0009c] In one aspect, there is provided a vector encoding one or more optimised gene products selected from *ispA, crtE, crtB* or *crtI* operably linked to a promoter.

[0009d] In one aspect, there is provided a vector encoding one or more optimised gene products selected from *crtY, blh* or *ybbO* operably linked to a promoter; optionally wherein the optimised gene products are selected from the group consisting of *crtY* and *blh,* and *crtY, blh* and *ybbO.*

[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.

[0010a] In one aspect, there is provided a system for producing an 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: a. *crtY* and *blh* for the production of retinal, or retinol, or a combination of retinal and retinol; or b. *crtY, blh* and *ybbO* for the production of 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.

[0011a] 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: a. *crtY* and *blh* operably linked to a promoter for the production of retinal, or retinol, or a combination of retinal and retinol; or b. *crtY, blh* and *ybbO* for the production of 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.

[0012a] In one aspect, there is provided a method for producing an apocarotenoid comprising the steps of: a. contacting a host cell as described herein 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.

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, wherein the apocarotenoid is retinal, or retinol, or a combination of retinal and retinol.

2. The method as claimed in claim 1, wherein said at least one optimised apocarotenoid generating enzyme is selected from *crtY, blh*, *ybbO*, or a combination thereof.

3. The method as claimed in claim 1, wherein the apocarotenoid is retinal, or retinol, or a combination of retinol and retinal, and wherein said at least one optimised apocarotenoid generating enzyme is selected from *crtY* and *blh*, and form an operon having the structure *crtY-blh.*

4. The method as claimed in claim 1, wherein the apocarotenoid is retinol and wherein said at least one optimised apocarotenoid generating enzyme is selected from *crtY* and *blh and ybbO*, and form an operon having the structure *crtY-blh-ybbO*.

5. The method as claimed in any one of claims 2 to 4, wherein said *crtY* is derived from *Pantoea ananatis*; optionally wherein said *blh* is derived from *Uncultured marine bacterium HF10_19P19* or *66A03*; wherein said *ybbO* is derived from *Escherichia coli* and optionally wherein the method further comprises screening for an expression level of retinal in an amount of up 10-1000 mg/L in a 24 hour period.

6. The method of any one of claims 1 to 5, 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.

7. The method as claimed in claim 6, wherein said one or more first gene products form an operon having the structure: *atoB-hmgS-thmgR*; optionally wherein said one or more first gene products form an operon having the structure: *crtY-atoB-hmgS-thmgR*; optionally wherein said one or more second gene products form an operon having the structure: *mevK-pmk-pmd-idi*; and optionally wherein said one or more third gene products form an operon having the structure: *crtE-crtB-crtI-ispA*.

8. The method of any of claims 1 to 7, wherein the host cell is *Escherichia coli* selected from BL21 or MG1655; optionally 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; and optionally wherein the optimisation of the apocarotenoid generating enzymes and optimised gene products is achieved by codon optimisation or site-directed mutagenesis.

9. 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,
wherein said at least one optimised apocarotenoid generating enzyme is selected from *crtY, blh*, *ybbO*, or a combination thereof.

10. The host cell as claimed in claim 9, wherein the optimised gene products are selected from the group consisting of *crtY* and *blh,* and *crtY, blh* and *ybbO*; and optionally wherein the host cell is *Escherichia coli* selected from BL21 or MG1655.

11. The host cell as claimed in any one of claims 9 or 10, 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.

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

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

14. A vector encoding one or more optimised gene products selected from *ispA, crtE, crtB* or *crtI* operably linked to a promoter.

15. A vector encoding one or more optimised gene products selected from *crtY, blh* or *ybbO* operably linked to a promoter; optionally wherein the optimised gene products are selected from the group consisting of *crtY* and *blh,* and *crtY, blh* and *ybbO*.

16. A system for producing an 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:

    a. *crtY* and *blh* for the production of retinal, or retinol, or a combination of retinal and retinol; or

    b. *crtY, blh* and *ybbO* for the production of retinol.

17. The system as claimed in claim 16, 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.

18. A kit when used in the method of any of claims 1 to 8, 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:

a. *crtY* and *blh* operably linked to a promoter for the production of retinal, or retinol, or a combination of retinal and retinol; or

b. *crtY, blh* and *ybbO* for the production of retinol.

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

a. contacting a host cell as claimed in any one of claims 9 to 11 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.

20. The method as claimed in claim 19, wherein the organic layer is coconut oil or soybean oil; optionally wherein the apocarotenoid is selected from retinal, or retinol, or a combination of retinal and retinol.