therefore exogenously supplied olivetolate presents a rate limiting step in the production of down-stream metabolites. Other aromatic prenyltransferase substrates such as divarinolic acid (DVA) encounter the same issues with respect to endogenous production, metabolic burden of heterologous production, and rate limiting membrane transport. Thus, there is a long felt and unmet need to develop a cost-effective heterologous system for the production of cannabinoids *in vivo*.

SUMMARY

[0006] Described herein are improved methods, compositions, and host cells for improved prenylation of aromatic substrates, or production of down-stream metabolites thereof, in a (e.g., prokaryotic) host cell. The present inventors have identified membrane transporters that are functional and capable of increasing the transport of extracellular aromatic prenyltranferase substrates such as olivetolate into the (e.g., prokaryotic) host cell when expressed as heterologous transporters in a host cell. For example, the present inventors have identified a major facilitator superfamily (MFS) aromatic acid antiporter that is functional and capable of increasing the transport of extracellular aromatic prenyltranferase substrates such as olivetolate into the (e.g., prokaryotic) host cell. Independently, the present inventors have identified an outer membrane porin (OMP) superfamily transporter that is functional and capable of increasing the transport of extracellular aromatic prenyltranferase substrates such as olivetolate into the (e.g., prokaryotic) host cell. Without wishing to be bound by theory, the present inventors hypothesize that the increased transport of aromatic prenyltransferase substrates such as olivetolate into the cell, e.g., via an antiporter or porin, increases flux through the aromatic prenylation step and thereby improves production of down-stream metabolic products. In some cases, the increased flux decreases the (e.g., steady state) intracellular concentration of toxic intermediates such as geranylpyrophosphate (GPP) and thereby improves production of down-stream metabolic products.

[0006a] In one aspect, the present disclosure refers to a host cell comprising:

a. an expression cassette comprising a promoter operably linked to a heterologous nucleic acid encoding a heterologous transporter or a functional fragment thereof, wherein the transporter is selected from the group consisting of a major facilitator superfamily (MFS) aromatic acid antiporter and an OprD family porin; and

b. an exogenous aromatic substrate selected from olivetolate, divarinolate, olivetolic acid, divarinolic acid (DVA), olivetol, divarinol, resveratrol, naringenin, phlorisovalerophenone, apigenin, diadzein, genestein, orcinol, and combinations thereof,

wherein said host cell is capable of increased import of the exogenous aromatic substrate into the host cell as compared to a control host cell that lacks the expression cassette of a

[0006b] In another aspect, the present disclosure refers to a method of producing a terpenoid, the method comprising culturing a host cell as described herein under conditions suitable to express the ispDE bifunctional enzyme, wherein the host cell comprises the ispDE bifunctional enzyme.

[0006c] In another aspect, the present disclosure refers to a method of increasing the transport of olivetolate into a prokaryotic host cell, the method comprising culturing a host cell as described herein in culture media containing exogenous aromatic substrate of the transporter under conditions suitable to express the transporter.

[0006d] In another aspect, the present disclosure refers to a method of prenylating olivetolate and/or DVA, the method comprising culturing a host cell as described herein in culture media containing exogenous olivetolate and/or DVA under conditions suitable to express the transporter and the aromatic prenyltransferase, thereby prenylating the olivetolate and/or DVA, wherein the host cell comprises the aromatic prenyltransferase.

[0007] Thus, the present disclosure provides a host cell comprising: a) an expression cassette comprising a promoter operably linked to a heterologous nucleic acid encoding a transporter; and, and b) an exogenous aromatic substrate of the transporter. In examples, the host cell is capable of increased import of an aromatic substrate of the transporter into the host cell as compared to a control prokaryotic host cell that lacks the expression cassette of a).

[0008] For example, in one example, the present disclosure provides a host cell comprising: a) an expression cassette comprising a promoter operably linked to a heterologous nucleic acid encoding a major facilitator superfamily (MFS) aromatic acid antiporter; and, and b) an exogenous aromatic substrate of the MFS aromatic acid antiporter. In examples, the host cell is capable of increased import of the aromatic substrate of the MFS aromatic acid antiporter into the host cell as compared to a control prokaryotic host cell that lacks the expression cassette of a). As another example, in one example, the present disclosure provides a host cell comprising: a) an expression cassette comprising a promoter operably linked to a heterologous nucleic acid encoding a OMP superfamily porin; and, and b) an

CLAIMS

What is claimed is:

1. A host cell comprising:
    a. an expression cassette comprising a promoter operably linked to a heterologous nucleic acid encoding a heterologous transporter or a functional fragment thereof, wherein the transporter is selected from the group consisting of a major facilitator superfamily (MFS) aromatic acid antiporter and an OprD family porin; and
    b. an exogenous aromatic substrate selected from olivetolate, divarinolate, olivetolic acid, divarinolic acid (DVA), olivetol, divarinol, resveratrol, naringenin, phlorisovalerophenone, apigenin, diadzein, genestein, orcinol, and combinations thereof,

    wherein said host cell is capable of increased import of the exogenous aromatic substrate into the host cell as compared to a control host cell that lacks the expression cassette of a).

2. The host cell of claim 1, wherein the cell is a prokaryote, preferably wherein the prokaryote is selected from the group consisting of a prokaryote of the genus *Escherichia*, *Panteoa*, *Bacillus*, *Corynebacterium*, or *Lactococcus*.

3. The host cell of claim 1, wherein the cell is *Escherichia coli (E. coli)*, *Panteoa citrea*, *C. glutamicum*, *Bacillus subtilis*, or *L. lactis*.

4. The host cell of claim 1, wherein the cell is *Escherichia coli (E. coli)*.

5. The host cell of any one of claims 1 - 4, wherein the transporter is the MFS aromatic acid antiporter pcaK or a functional fragment thereof; or wherein the transporter is the OprD family porin pp3656 or a functional fragment thereof.

6. The host cell of any one of claims 1 - 5, wherein the transporter is at least 80% identical to, or identical to, 100 contiguous amino acids of the sequence set forth in: SEQ ID NO. 6, 7, 8, or 9, wherein the transporter is capable of increasing the transport of the exogenous aromatic substrate into the host cell.

7. The host cell of any one of claims 1-6, wherein the host cell further comprises a heterologous aromatic prenyltransferase or functional fragment thereof, wherein the aromatic prenyltransferase is functional and capable of prenylating the aromatic acid substrate.

8. The host cell of claim 7, wherein the heterologous aromatic prenyltransferase is CBGAS or NphB or a functional fragment thereof.

9. The host cell of claim 8, wherein the heterologous aromatic prenyltransferase is a functional fragment of CBGAS.

10. The host cell of claim 9, wherein the functional fragment of CBGAS is at least 80% identical to, or identical to, 100 contiguous amino acids of the sequence set forth in SEQ ID NO. 3, wherein the functional fragment of CBGAS is capable of prenylating the exogenous aromatic acid substrate.

11. The host cell of any one of claims 1-10, wherein the host cell further comprises an expression cassette comprising a promoter operably linked to a nucleic acid encoding one or more MEP pathway enzymes selected from the group consisting of dxs, ispC, ispD, ispE, ispF, ispDF, ispG, ispH, and idi, or a that is at least 90%, 95%, or 99% identical to a respective native prokaryotic sequence, wherein the one or more MEP pathway enzymes have the same activity as the respective native prokaryotic sequence.

12. The host cell of claim 11, wherein said one or more MEP pathway enzymes comprise:
    a. dxs, ispF and idi, or
    b. dxs, ispDF, and idi.

13. The host cell of any one of claims 1-11, wherein the host cell further comprises an expression cassette selected from the group consisting of: an expression cassette comprising a promoter operably linked to a nucleic acid encoding ispDF, an expression cassette comprising a promoter operably linked to a nucleic acid encoding ispDE and an expression cassette comprising a promoter operably linked to a nucleic acid encoding ispEF.

14. The host cell of claim 12, wherein the host cell comprises:

a. a heterologous nucleic acid encoding ispDF and a heterologous nucleic acid encoding ispE;

b. a heterologous nucleic acid encoding ispDE and heterologous nucleic acid encoding ispF; or

c. a heterologous nucleic acid encoding ispEF and a heterologous nucleic acid encoding ispD.

15. The host cell of claim 13, wherein the bifunctional ispDE enzyme comprises a sequence at least 80% identical to the sequence set forth in SEQ ID NO:10, wherein the bifunctional ispDE enzyme exhibits ispD activity and ispE activity.

16. The host cell of any one of claims 11-15, wherein the host cell further comprises a heterologous expression cassette comprising a promoter operably linked to at least one prokaryotic chaperone.

17. The host cell of claim 11-16, wherein the host cell further comprises an expression cassette comprising a promoter operably linked to a nucleic acid encoding a terpenoid synthase.

18. The host cell of any one of claims 1-11, and 13, wherein the host cell further comprises an expression cassette comprising a promoter operably linked to a nucleic acid encoding GPP synthase.

19. The host cell of any one of claims 1-11, 13 and 18, wherein the host cell is in a culture medium comprising olivetolate, DVA, olivetol, or divarinol, preferably wherein the host cell is in a culture medium comprising olivetolate and/or DVA.

20. The host cell of any one of claims 1-11, 13, and 18-19, wherein the host cell further comprises an expression cassette comprising a promoter operably linked to a nucleic acid encoding a cannabinoid synthase, an aromatic prenyltransferase, a GPP synthase, and combinations thereof.

21. The host cell of claim 20, wherein the cannabinoid synthase is a CBDA synthase, CBCA synthase, or THCA synthase, preferably wherein the cannabinoid synthase is a CBDA synthase.

22. The host cell of any one of claims 11 to 21, wherein at least one, at least two, at least three, at least four, or all heterologous expression cassettes are integrated into the genome of the host cell.

23. The host cell of any one of claims 11 to 21, wherein at least one of the expression cassettes is not integrated into the genome of the host cell.

24. A method of producing a terpenoid, the method comprising culturing a host cell of any one of claims 11 to 23 under conditions suitable to express the ispDE bifunctional enzyme, wherein the host cell comprises the ispDE bifunctional enzyme.

25. The method of claim 24, wherein the method comprises culturing the host cell in culture media comprising an exogenously supplied substrate of a heterologously expressed aromatic prenyltransferase.

26. The method of claim 25, wherein the exogenously supplied substrate comprises olivetolate or divarinolic acid, preferably olivetolate.

27. A method of increasing the transport of olivetolate into a prokaryotic host cell, the method comprising culturing a host cell according to any one of claims 1-11, 13, and 18-21 in culture media containing exogenous aromatic substrate of the transporter under conditions suitable to express the transporter.

28. A method of prenylating olivetolate and/or DVA, the method comprising culturing a host cell according to any one of claims 7-11, 13, and 18-21 in culture media containing exogenous olivetolate and/or DVA under conditions suitable to express the transporter and the aromatic prenyltransferase, thereby prenylating the olivetolate and/or DVA, wherein the host cell comprises the aromatic prenyltransferase.

29. The method of claim 28, wherein the aromatic prenyltransferase is a geranyl-diphosphate:olivetolate geranyltransferase, and the method comprises producing cannabigerolic acid.

30. The method of any one of claims 28-29, wherein the method increases the production of a prenylated olivetolate or DVA product as compared to a control method performed under conditions that do not express, or express a lower amount or activity of, the transporter.

31. The method of any one of claims 28-30, wherein the method comprises harvesting and lysing the cultured cell, thereby producing cell lysate.

32. The method of claim 31, wherein the method comprises purifying the prenylated olivetolate or DVA product, from the cell lysate.

33. The method of any one of claims 28-30, wherein the method comprises harvesting spent culture medium produced by culturing the host cell.

34. The method of claim 33, wherein the method comprises purifying the prenylated olivetolate or DVA product, from the spent culture medium.

35. The method of claim 32 or 34, wherein the method comprises purifying CBGA, or a decarboxylation product thereof, from the cell lysate or spent culture medium.

36. The method of claim 30 or 34, wherein the method comprises purifying CBDA, or a decarboxylation product thereof, from the cell lysate or spent culture medium.