

CLAIMS

What is claimed is:

1. Cells in culture capable of producing isoprene wherein the cells comprises a heterologous nucleic acid encoding an isoprene synthase polypeptide and are in stationary phase and wherein the isoprene production is greater than or 2-fold more than the amount of isoprene produced during growth phase for the same length of time.
2. The cells of claim 1 wherein the isoprene is produced in the gas phase and
 - (a) wherein the gas phase comprises greater than or about 9.5 % (volume) oxygen, and the concentration of isoprene in the gas phase is less than the lower flammability limit or greater than the upper flammability limit or
 - (b) the concentration of isoprene in the gas phase is less than the lower flammability limit or greater than the upper flammability limit, and the cells produce greater than about 400 nmole/g_{wcm}/hr of isoprene.
3. Cells in culture that produce greater than about 400 nmole/g_{wcm}/hr of isoprene wherein the cells are grown under conditions that decouple isoprene production from cell growth.
4. Cells in culture that have an average volumetric productivity of isoprene greater than about 0.1 mg/L_{broth}/hr wherein the cells are grown under conditions that decouple isoprene production from cell growth.
5. Cells in culture that convert more than about 0.002 molar percent of the carbon that the cells consume from a cell culture medium into isoprene wherein the cells are grown under conditions that decouple isoprene production from cell growth.
6. The cells of any one of claims 1-5 wherein the cells are grown under limited glucose conditions.

7. The cells of any one of claims 1-6 wherein the amount of isoprene produced during stationary phase is greater than or 2, 3, 4, 5, 10, 20, 30, 40, 50 times the amount of isoprene produced during the growth phase for the same length of time.

8. A composition comprising (i) a gas phase that comprises isoprene and (ii) cells in culture of any one of claims 1-7.

9. The composition of claim 8 wherein the composition comprises a closed system, and the gas phase comprises greater than or 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 ug/L of isoprene when normalized to 1 mL of 1 OD₆₀₀ cultured for 1 hour.

10. The composition of claim 8 wherein the composition comprises an open system, and the gas phase comprises greater than or 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 ug/L of isoprene when sparged at a rate of 1 vvm.

11. The composition of claim 8 wherein the composition comprises a volatile organic fraction of the gas phase comprising greater than or 99.90, 99.92, 99.94, 99.96, 99.98, or 100% isoprene by weight compared to the total weight of all C5 hydrocarbons in the volatile organic fraction.

12. A method of producing isoprene, the method comprising

(a) culturing cells of any one of claims 1-7 under suitable culture conditions for the production of isoprene; wherein the amount of isoprene produced during stationary phase is greater than or 2-fold more than the amount of isoprene produced during growth phase for the same length of time, and

(b) producing isoprene.

13. The method of claim 12 wherein the cells are cultured under limited glucose conditions.

14. The method of claim 12 wherein the isoprene is recovered from an off-gas portion of the cell culture.

15. A method of producing isoprene, the method comprising:
- (a) culturing cells under suitable culture conditions for the production of isoprene, wherein the gas phase comprises greater than or 9.5 % (volume) oxygen, and
 - (b) producing isoprene, wherein the concentration of isoprene in the gas phase is less than the lower flammability limit or greater than the upper flammability limit, and wherein the cells produce greater than 400 nmole/g_{wcm}/hr of isoprene.
16. A method of producing isoprene, the method comprising
- (a) culturing cells comprising a heterologous nucleic acid encoding an isoprene synthase polypeptide under suitable culture conditions for the production of isoprene, wherein the gas phase comprises less than or 9.5 % (volume) oxygen, and
 - (b) producing isoprene in the gas phase, wherein the gas phase comprises a nonflammable concentration of isoprene and less than or about 9.5 % (volume) oxygen, and wherein the cells produce greater than 400 nmole/g_{wcm}/hr of isoprene.
17. The method of claim 16, wherein the concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
18. The method of claim 17, wherein the LFL of isoprene is from 1.5 vol. % to 2.0 vol. %.
19. The method of claim 16, wherein the concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.
20. The method of claim 19, wherein the UFL of isoprene is from 2.0 vol. % to 12.0 vol. %.
21. The method of claim 16, wherein isoprene is produced in the gas phase at a temperature between 25 °C to 55°C.

22. The method of claim 16, wherein isoprene is produced in the gas phase at a pressure between 1 atmosphere and 3 atmospheres.

23. The method of claim 16, wherein the isoprene synthase polypeptide is a plant isoprene synthase polypeptide.

24. The method of claim 16, wherein the cells are bacterial cells.

25. The method of claim 24, wherein the bacterial cells are selected from the group consisting of *E. coli*, *P. citrea*, *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. clausii*, *B. halodurans*, *B. megaterium*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. thuringiensis*, *S. lividans*, *S. coelicolor*, *S. griseus*, *Pseudomonas sp.*, and *P. alcaligenes* cells.

26. The method of claim 16, wherein the cells are fungal cells.

27. The method of claim 26, wherein the fungal cells are selected from the group consisting of *Aspergillus sp.*, yeast, *Trichoderma sp.*, *Yarrowia sp.*, *Saccharomyces sp.*, *Schizosaccharomyces sp.*, *Pichia sp.*, *Candida sp.*, *A. oryzae*, *A. niger*, *S. cerevisiae*, *S. pombe*, *T. reesei*, *H. insolens*, *H. lanuginosa*, *H. grisea*, *C. lucknowense*, *A. sojae*, *A. japonicus*, *A. nidulans*, *A. aculeatus*, *A. awamori*, *F. roseum*, *F. gramineum*, *F. cerealis*, *F. oxysporum*, *F. venenatum*, *N. crassa*, *M. miehei*, *T. viride*, *F. oxysporum*, and *F. solani* cells.

28. The method of claim 16, wherein the cells are algal cells.

29. The method of claim 28, wherein the algal cells are selected from the group consisting of green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, and dinoflagellates

30. The method of claim 16, wherein the cells further comprise a nucleic acid encoding an isopentyl-diphosphate delta-isomerase (IDI) polypeptide.

31. The method of claim 30, wherein the nucleic acid encoding the IDI polypeptide is a heterologous nucleic acid.

32. The method of claim 30, wherein the IDI polypeptide is a yeast IDI polypeptide.

33. The method of claim 30, wherein the nucleic acid encoding the IDI polypeptide is a copy of an endogenous nucleic acid encoding an IDI polypeptide.

34. The method of claim 16, wherein the cells further comprise a heterologous nucleic acid encoding a 1-deoxyxylulose-5-phosphate synthase (DXS) polypeptide.

35. The method of claim 16, wherein the cells further comprise a copy of an endogenous nucleic acid encoding a DXS polypeptide.

36. The method of claim 16, wherein the cells further comprise one or more heterologous nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides.

37. The method of claim 16, wherein the cells further comprise one or more copies of endogenous nucleic acids encoding one or more MVA pathway polypeptides.

38. The method of claim 16, wherein the cells further comprise one or more nucleic acids encoding the polypeptides of the entire MVA pathway.

39. The method of claim 16, wherein the cells further comprise:
(a) a nucleic acid encoding an isopentyl-diphosphate delta-isomerase (IDI) polypeptide; and

(b) (i) a nucleic acid encoding a 1-deoxyxylulose-5-phosphate synthase (DXS) polypeptide and/or (ii) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides.

40. The method of claim 39, wherein the nucleic acid encoding a DXS polypeptide is a heterologous nucleic acid.

41. The method of claim 39, wherein the nucleic acid encoding a DXS polypeptide is a copy of an endogenous nucleic acid.

42. The method of claim 39, wherein said one or more nucleic acids encoding one or more MVA pathway polypeptides are heterologous nucleic acids.

43. The method of claim 39, wherein said one or more nucleic acids encoding one or more MVA pathway polypeptides are endogenous nucleic acids.

CLAIMS

What is claimed is:

1. Cells in a culture system capable of producing isoprene, wherein the cells comprise a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the system comprises a gas phase comprising a nonflammable concentration of isoprene, and wherein the cells produce greater than about 400 nmole/g_{wcm}/hr of isoprene.
2. The culture system of claim 1, wherein the gas phase further comprises less than or about 9.5% (volume) oxygen.
3. The culture system of claim 2, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
4. The culture system of claim 3, wherein the LFL of isoprene is from about 1.5 (volume) % to about 2.0 (volume) %.
5. The culture system of claim 2, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.
6. The culture system of claim 5, wherein the UFL of isoprene is from about 2.0 (volume) % to about 12.0 (volume) %.
7. The culture system of claim 1, wherein the gas phase further comprises greater than or about 9.5% (volume) oxygen.
8. The culture system of claim 7, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
9. The culture system of claim 8, wherein the LFL of isoprene is less than about 1.5 (volume) %.
10. The culture system of claim 7, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.

11. The culture system of claim 10, wherein the UFL of isoprene is greater than about 12.0 (volume) %.
12. The culture system of any one of claims 1-11, wherein the cells are cultured under conditions that decouple isoprene production from cell growth.
13. The culture system of any one of claims 1-12, wherein the cells have an average volumetric productivity of isoprene greater than about 0.1 mg/L_{broth}/hr.
14. The culture system of any one of claims 1-13, wherein the cells convert more than about 0.002 molar percent of the carbon that the cells consume from a cell culture medium into isoprene.
15. The culture system of any one of claims 1-15, wherein the cells are grown under limited glucose conditions.
16. The culture system of any one of claims 1-15, wherein the cells producing isoprene produce an amount of isoprene during stationary phase that is greater than or about 2, 3, 4, 5, 10, 20, 30, 40, 50 times the amount of isoprene produced during the growth phase for the same length of time.
17. The culture system of any one of claims 1-16, wherein the gas phase comprising a nonflammable concentration of isoprene comprises a volatile organic fraction comprising greater than or about 99.90, 99.92, 99.94, 99.96, 99.98, or 100% isoprene by weight compared to the total weight of all C5 hydrocarbons in the volatile organic fraction.
18. The culture system of any one of claims 1-17, wherein the gas phase comprising a nonflammable concentration of isoprene comprises a volatile organic fraction comprising less than or about 0.12, 0.10, 0.08, 0.06, 0.04, 0.02, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005, or 0.00001% C5 hydrocarbons other than isoprene.
19. A method of producing isoprene, the method comprising
 - (a) culturing recombinant cells comprising a heterologous nucleic acid encoding an isoprene synthase polypeptide under suitable culture conditions for the production of isoprene in a gas phase; and

- (b) producing isoprene in the gas phase, wherein the gas phase comprises a nonflammable concentration of isoprene and less than or about 9.5 % (volume) oxygen, and wherein the cells produce greater than about 400 nmole/g_{wcm}/hr of isoprene.
20. A method of producing isoprene, the method comprising
- (a) culturing recombinant cells comprising a heterologous nucleic acid encoding an isoprene synthase polypeptide under suitable culture conditions for the production of isoprene in a gas phase; and
 - (b) producing isoprene in the gas phase, wherein the gas phase comprises a nonflammable concentration of isoprene and greater than or about 9.5 % (volume) oxygen, and wherein the cells produce greater than about 400 nmole/g_{wcm}/hr of isoprene.
21. The method of claim 19, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
22. The method of claim 21, wherein the LFL of isoprene is from about 1.5 (volume) % to about 2.0 (volume) %.
23. The method of claim 19, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.
24. The method of claim 23, wherein the UFL of isoprene is from about 2.0 (volume) % to about 12.0 (volume) %.
25. The method of claim 20, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
26. The method of claim 25, wherein the LFL of isoprene is less than about 1.5 (volume) %.
27. The method of claim 20, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.

28. The method of claim 27, wherein the UFL of isoprene is greater than about 12.0 (volume) %.
29. The method of claim 19 or 20, wherein isoprene is produced in the gas phase at a temperature between about 25°C to about 55°C.
30. The method of claim 19 or 20, wherein isoprene is produced in the gas phase at a pressure between about 1 atmosphere and 3 atmospheres.
31. The method of claim 19 or 20, wherein the isoprene synthase polypeptide is a plant isoprene synthase polypeptide.
32. The method of claim 19 or 20, wherein the cells are cultured under limited glucose conditions.
33. The method of claim 19 or 20, wherein the isoprene is recovered from an off-gas portion of the culture.
34. The method of claim 19 or 20, wherein the cells are bacterial cells.
35. The method of claim 34, wherein the bacterial cells are selected from the group consisting of *Escherichia coli*, *Pantoea citrea*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus clausii*, *Bacillus halodurans*, *Bacillus megaterium*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus thuringiensis*, *Streptomyces lividans*, *Streptomyces coelicolor*, *Streptomyces griseus*, *Pseudomonas* sp., and *Pseudomonas alcaligenes* cells.
36. The method of claim 19 or 20, wherein the cells are fungal cells.
37. The method of claim 36, wherein the fungal cells are selected from the group consisting of *Aspergillus* sp., yeast, *Trichoderma* sp., *Yarrowia* sp., *Saccharomyces* sp., *Schizosaccharomyces* sp., *Pichia* sp., *Candida* sp., *Aspergillus oryzae*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Trichoderma reesei*, *H. insolens*, *H. lanuginosa*, *H. grisea*, *Candida lucknowense*, *Aspergillus sojae*, *Aspergillus japonicus*,

Aspergillus nidulans, *Aspergillus aculeatus*, *Aspergillus awamori*, *Fusarium roseum*, *Fusarium gramineum*, *Fusarium cerealis*, *Fusarium oxysporum*, *F. venenatum*, *Neurospora crassa*, *Mucor miehei*, *Trichoderma viride*, *Fusarium oxysporum*, and *Fusarium solani* cells.

38. The method of claim 19 or 20, wherein the cells are algal cells.
39. The method of claim 38, wherein the algal cells are selected from the group consisting of green algae, red algae, glaucophytes, chlorarachniophytes, euglenids, chromista, and dinoflagellates.
40. The method of claim 19 or 20, wherein the cells further comprise a nucleic acid encoding an isopentyl-diphosphate delta-isomerase (IDI) polypeptide.
41. The method of claim 40, wherein the nucleic acid encoding the IDI polypeptide is a heterologous nucleic acid.
42. The method of claim 40, wherein the IDI polypeptide is a yeast IDI polypeptide.
43. The method of claim 40, wherein the nucleic acid encoding the IDI polypeptide is a copy of an endogenous nucleic acid encoding an IDI polypeptide.
44. The method of claim 19 or 20, wherein the cells further comprise a heterologous nucleic acid encoding a 1-deoxyxylulose-5-phosphate synthase (DXS) polypeptide.
45. The method of claim 19 or 20, wherein the cells further comprise a copy of an endogenous nucleic acid encoding a DXS polypeptide.
46. The method of claim 19 or 20, wherein the cells further comprise one or more heterologous nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides.
47. The method of claim 19 or 20, wherein the cells further comprise one or more copies of endogenous nucleic acids encoding one or more MVA pathway polypeptides.

48. The method of claim 19 or 20, wherein the cells further comprise one or more nucleic acids encoding the polypeptides of the entire MVA pathway.
49. The method of claim 19 or 20, wherein the cells further comprise:
 - (a) a nucleic acid encoding an isopentyl-diphosphate delta-isomerase (IDI) polypeptide; and,
 - (b) (b)(i) a nucleic acid encoding a 1-deoxyxylulose-5-phosphate synthase (DXS) polypeptide and/or (ii) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides.
50. The method of claim 49, wherein the nucleic acid encoding a DXS polypeptide is a heterologous nucleic acid.
51. The method of claim 49, wherein the nucleic acid encoding a DXS polypeptide is a copy of an endogenous nucleic acid.
52. The method of claim 49, wherein said one or more nucleic acids encoding one or more MVA pathway polypeptides are heterologous nucleic acids.
53. The method of claim 49, wherein said one or more nucleic acids encoding one or more MVA pathway polypeptides are endogenous nucleic acids.
54. A system comprising cells in culture capable of producing isoprene, wherein the cells comprise a heterologous nucleic acid encoding an isoprene synthase polypeptide, wherein the system comprises a gas phase comprising a nonflammable concentration of isoprene, and wherein the cells produce greater than about 400 nmole/g_{wcm}/hr of isoprene.
55. The system of claim 54, wherein the gas phase further comprises less than or about 9.5% (volume) oxygen.
56. The system of claim 55, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
57. The system of claim 56, wherein the LFL of isoprene is from about 1.5 (volume) % to about 2.0 (volume) %.

58. The system of claim 55, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.
59. The system of claim 58, wherein the UFL of isoprene is from about 2.0 (volume) % to about 12.0 (volume) %.
60. The system of claim 54, wherein the gas phase further comprises greater than or about 9.5% (volume) oxygen.
61. The system of claim 60, wherein the nonflammable concentration of isoprene in the gas phase is less than the lower flammability limit (LFL) of isoprene.
62. The cells in culture of claim 61, wherein the LFL of isoprene is less than about 1.5 (volume) %.
63. The cells in culture of claim 60, wherein the nonflammable concentration of isoprene in the gas phase is greater than the upper flammability limit (UFL) of isoprene.
64. The cells in culture of claim 61, wherein the UFL of isoprene is greater than about 12.0 (volume) %.
65. The cells in culture of any one of claims 54-64, wherein the cells are cultured under conditions that decouple isoprene production from cell growth.
66. The cells in culture of any one of claims 54-65, wherein the cells have an average volumetric productivity of isoprene greater than about 0.1 mg/L_{broth}/hr.
67. The cells in culture of any one of claims 54-66, wherein the cells convert more than about 0.002 molar percent of the carbon that the cells consume from a cell culture medium into isoprene.
68. The cells in culture of any one of claims 54-67, wherein the cells are grown under limited glucose conditions.

69. The cells in culture of any one of claims 54-67, wherein the cells producing isoprene produce an amount of isoprene during stationary phase that is greater than or about 2, 3, 4, 5, 10, 20, 30, 40, 50 times the amount of isoprene produced during the growth phase for the same length of time.
70. The cells in culture of any one of claims 54-70, wherein the gas phase comprising a nonflammable concentration of isoprene comprises a volatile organic fraction comprising greater than or about 99.90, 99.92, 99.94, 99.96, 99.98, or 100% isoprene by weight compared to the total weight of all C5 hydrocarbons in the volatile organic fraction.
71. The cells in culture of any one of claims 54-70, wherein the gas phase comprising a nonflammable concentration of isoprene comprises a volatile organic fraction comprising less than or about 0.12, 0.10, 0.08, 0.06, 0.04, 0.02, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005, or 0.00001% C5 hydrocarbons other than isoprene.