Claims

1. A method of producing cis-α-irone, the method comprises providing an enzyme capable of converting psi-ionone to cis-α-irone; and contacting the enzyme and psi-ionone under suitable conditions to produce cis-α-irone,

wherein the enzyme is selected from the group consisting of a modified enzyme, SEQ ID No. 1, and SEQ ID No. 3, the modified enzyme consists essentially of a first substitution of base SEQ ID No. 3 at a position selected from the group consisting of position 200, position 180, position 160, and position 236,

wherein if the first substitution is at position 200, the first substitution is selected from the group consisting of phenylalanine, isoleucine, leucine, valine, and tryptophan,

wherein if the first substitution is at position 180, the first substitution is selected from the group consisting of alanine, cysteine, glutamic acid, isoleucine, methionine, and valine,

wherein if the first substitution is at position 160, the first substitution is selected from the group consisting of alanine, cysteine, histidine, isoleucine, leucine, methionine, asparagine, glutamine, threonine, valine, and tyrosine,

wherein if the first substitution is at position 236, the first substitution is selected from the group consisting of cysteine, glutamic acid, histidine, isoleucine, leucine, asparagine, serine, threonine, and valine.

2. The method according to claim 1, the modified enzyme further consists essentially of a second substitution selected from the group consisting of position 182 and position 180, wherein if the second substitution is at position 182, the second substitution is selected from the group consisting of glutamic acid, threonine, asparagine and glutamine, wherein the second substitution may be selected at position 180 only if the first substitution is not at position 180, and if the second substitution is at position 180, the second substitution is selected from the group consisting of alanine, cysteine.

3. The method according to claim 1 or claim 2, the modified enzyme further consists essentially of a third substitution at position 273, wherein the third substitution is selected from the group consisting of valine, isoleucine, and lysine.

4. The method according to any one of claims 1 to 3, the modified enzyme further consists essentially of a fourth substitution at position 180, wherein the first

substitution is not at position 180 and the second substitution if present is not at position 180, and the fourth substitution is alanine or cysteine.

5. The method according to any one of claims 1 to 4, the modified enzyme further consists essentially of a fifth substitution at position 202 to a bulkier amino acid.

6. The method according to claim 5 wherein the fifth substitution is selected from the group consisting of leucine, valine, and phenylalanine.

7. The method according to any one of claims 1 to 6, the modified enzyme further consists essentially ofa sixth substitution at position 65, wherein the sixth substitution is selected from the group consisting of phenylalanine, leucine, and methionine.

8. The method according to any one of claims 1 to 7, the modified enzyme further consists essentially of a seventh substitution at position 156, wherein the seventh substitution is selected from the group consisting of aspartic acid, alanine, proline, glycine, and serine.

9. The method according to any one of claims 1 to 8, the modified enzyme further consists essentially ofan eighth substitution at position 91, wherein the eighth substitution is proline.

10. The method according to any one of claims 1 to 9, the modified enzyme further consists essentially of a ninth substitution at position 231, wherein the ninth substitution is aspartic acid.

11. The method according to any one of claims 1 to 10, the modified enzyme further consists essentially ofa tenth substitution selected from the group consisting of alanine at position 244, alanine at position 245, and proline at position 267.

12. The method according to any one of claims 1 to 11, the modified enzyme further consists essentially of an eleventh substitution at position 197, wherein the eleventh substitution is arginine.

13. The method according to any one of claims 1 to 12, the modified enzyme further consists essentially of a twelfth substitution at position 60, wherein the twelfth substitution is selected from the group consisting of valine, lysine, and arginine.

14. The method according to any one of claims 1 to 13, the modified enzyme further consists essentially ofa thirteenth substitution selected from the group consisting of:

(i) the thirteenth substitution is at position 11 and selected from histidine, leucine, proline, methionine, valine, and tryptophan;

(ii) the thirteenth substitution is at position 12 and selected from lysine, alanine glycine, and arginine;

(iii) the thirteenth substitution is at position 13 and selected from leucine, methionine, glutamine, alanine, and glycine;

(iv) the thirteenth substitution is at position 14 and selected from methionine, arginine, glycine, proline, leucine, and threonine;

(v) the thirteenth substitution is at position 94 and selected from arginine, and valine;

(vi) the thirteenth substitution is at position 95 and selected from isoleucine, cysteine, valine, and leucine;

(vii) the thirteenth substitution is at position 107 and selected from serine and glycine;

(viii) the thirteenth substitution is at position 123 and selected from asparagine, glutamine, and serine;

(ix) the thirteenth substitution is at position 126 and selected from serine and glutamic acid;

(x) the thirteenth substitution is at position 127 and selected from glycine and threonine;

(xi) the thirteenth substitution is at position 129 and selected from cysteine, lysine, and valine;

(xii) the thirteenth substitution is at position 137 and selected from glycine, alanine, aspartic acid, histidine, asparagine, serine, and threonine;

(xiii) the thirteenth substitution is at position 159 and is leucine;

(xiv) the thirteenth substitution is at position 176 and selected from leucine, valine, lysine, arginine and tyrosine, and optionally a fourteenth substitution of valine at position 248;

(xv) the thirteenth substitution is at position 185 and selected from valine and leucine;

(xvi) the thirteenth substitution is at position 190 and selected from glutamine, alanine and proline, and optionally a fifteenth substitution of serine at position 122;

(xvii) the thirteenth substitution is at position 191 and selected from serine, valine, leucine, isoleucine, tyrosine, and lysine;

(xviii) the thirteenth substitution is at position 192 and selected from serine;

(xix) the thirteenth substitution is at position 195 and selected from isoleucine and valine;

(xx) the thirteenth substitution is at position 212 and is leucine;

(xxi) the thirteenth substitution is at position 268 and selected from lysine, glutamine, histidine, arginine, and leucine;

(xxii) the thirteenth substitution is at position 269 and selected from leucine, glycine, arginine, and tryptophan; and

(xxiii) the thirteenth substitution is at position 272 and is alanine.

15. The method according to claim 1 wherein the modified enzyme comprises a sequence selected from the group consisting of SEQ ID No. 15, SEQ ID No. 14, SEQ ID No. 13, SEQ ID No. 16, SEQ ID No. 12, SEQ ID No. 11, SEQ ID No. 10, SEQ ID No. 9, SEQ ID No. 8, SEQ ID No. 7, SEQ ID No. 6, SEQ ID No. 5, SEQ ID No. 4, and SEQ ID No. 20 to 100.

16. The method according to any one of claims 1 to 15, the modified enzyme comprising a polyhistidine-tag.

17. The method according to any one of claims 1 to 16 wherein contacting the modified enzyme and psi-ionone is done in the presence of an auxiliary enzyme that removes or recycles S-adenosyl-L-homocysteine (SAH).

18. The method according to claim 17 wherein the auxiliary enzyme is selected from the group consisting of S-adenosylmethionine synthase (MetK), adenosylhomocysteinase (SAH1), 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase (mtn), 5-methylthioadenosine/S-adenosylhomocysteine deaminase (mtaD), and halide methyl transferase (HMT).

19. The method according to any one of claims 1 to 18 wherein psi-ionone has a maximum concentration of 5 mM.

20. The method according to any one of claims 1 to 19 wherein the modified enzyme is provided as a lysate of a cell expressing the modified enzyme or a host cell comprising a plurality of nucleic acid sequences to encode at least one host cell enzyme and a first nucleic acid sequence encoding the modified enzyme, wherein the plurality of enzymes are produced by the host cell to assist in converting glucose or glycerol to psi-ionone.

21. The method according to claim 20 wherein the host cell enzyme includes at least one of the following: HMG-CoA synthase (HmgS), Acetoacetyl-CoA thiolase (AtoB), truncated HMG-CoA reductase (tHmgR) or HMG-CoA reductase, mevalonate kinase (MevK), phosphomevalonate kinase (PMK), mevalonate pyrophosphate decarboxylase (PMD), IPP isomerase (Idi), GGPP synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI), FPP synthase (IspA), and modified OfCCD1 enzyme or OfCCD1 enzyme.

22. The method according to claim 20 or claim 21, wherein at least one of the following conditions are fulfilled:

(i) the host cell further comprises a second nucleic acid sequence to encode SAM cycle enzymes;

(ii) a third nucleic acid sequence encoding a transcription regulator metJ is absent in the host cell;

(iii) the host cell further comprises at least one T7 promoter sequence;

(iv) the host cell is *Escherichia coli*.

23. The method according to any one of claims 20 to 22 wherein the modified enzyme is provided by the host cell and the suitable conditions include a dissolved oxygen content of 30% or less, preferably 1% to 15%, more preferably 2% to 10%.

24. The method according to any one of claims 1 to 23 wherein at least 0.1 µg/L of cis-α-irone is produced or a minimum cis-to-trans α-irone ratio of 2 to 5.

25. A modified enzyme consists essentially of a first substitution of base SEQ ID No. 3 at a position selected from the group consisting of position 200, position 180, position 160, and position 236,

wherein if the first substitution is at position 200, the first substitution is selected from the group consisting of phenylalanine, isoleucine, leucine, valine, and tryptophan,

wherein if the first substitution is at position 180, the first substitution is selected from the group consisting of alanine, cysteine, glutamic acid, isoleucine, methionine, and valine,

wherein if the first substitution is at position 160, the first substitution is selected from the group consisting of alanine, cysteine, histidine, isoleucine, leucine, methionine, asparagine, glutamine, threonine, valine, and tyrosine,

wherein if the first substitution at position 236 is selected, the first substitution is selected from the group consisting of cysteine, glutamic acid, histidine, isoleucine, leucine, asparagine, serine, threonine, and valine.

26. The modified enzyme according to claim 25 further consists essentially of a second substitution selected from the group consisting of position 182 and position 180, wherein if the second substitution is at position 182, the second substitution is selected from the group consisting of glutamic acid, threonine, asparagine and glutamine, wherein the second substitution may be selected at position 180 only if the first substitution is not at position 180, and if the second substitution is at position 180, the second substitution is selected from the group consisting of alanine, and cysteine.

27. The modified enzyme according to claim 25 or claim 26 further consists essentially of a third substitution at position 273, wherein the third substitution is selected from the group consisting of valine, isoleucine, and lysine.

28. The modified enzyme according to any one of claims 25 to 27 further consists essentially of a fourth substitution at position 180, wherein the first substitution is not at position 180 and the second substitution if present is not at position 180, and the fourth substitution is alanine or cysteine.

29. The modified enzyme according to any one of claims 25 to 28 further consists essentially of a fifth substitution at position 202 to a bulkier amino acid.

30. The modified enzyme according to claim 29 wherein the fifth substitution is selected from the group consisting of leucine, valine, and phenylalanine.

31. The modified enzyme according to any one of claims 25 to 30 further consists essentially of a sixth substitution at position 65, wherein the sixth substitution is selected from the group consisting of phenylalanine, leucine, and methionine.

32. The modified enzyme according to any one of claims 25 to 31 further consists essentially of a seventh substitution at position 156, wherein the seventh substitution is selected from the group consisting of aspartic acid, alanine, proline, glycine, and serine.

33. The modified enzyme according to any one of claims 25 to 32 further consists essentially of an eighth substitution at position 91, wherein the eighth substitution is proline.

34. The modified enzyme according to any one of claims 25 to 33, the modified enzyme comprising a ninth substitution at position 231, wherein the ninth substitution is aspartic acid.

35. The modified enzyme according to any one of claims 25 to 34, the modified enzyme further consists essentially of a tenth substitution selected from the group consisting of alanine at position 244, alanine at position 245, and proline at position 267.

36. The modified enzyme according to any one of claims 25 to 35, the modified enzyme further consists essentially of an eleventh substitution at position 197, wherein the eleventh substitution is arginine.

37. The modified enzyme according to any one of claims 25 to 36, the modified enzyme further consists essentially ofa twelfth substitution at position 60, wherein the twelfth substitution is selected from the group consisting of valine, lysine, and arginine.

38. The modified enzyme according to any one of claims 25 to 37, the modified enzyme further consists essentially of a thirteenth substitution selected from the group consisting of:

(i) the thirteenth substitution is at position 11 and selected from histidine, leucine, proline, methionine, valine, and tryptophan;

(ii) the thirteenth substitution is at position 12 and selected from lysine, alanine, glycine, and arginine;

(iii) the thirteenth substitution is at position 13 and selected from leucine, methionine, glutamine, alanine, and glycine;

(iv) the thirteenth substitution is at position 14 and selected from methionine, arginine, glycine, proline, leucine, and threonine;

(v) the thirteenth substitution is at position 94 and selected from arginine, and valine;

(vi) the thirteenth substitution is at position 95 and selected from isoleucine, cysteine, valine, and leucine;

(vii) the thirteenth substitution is at position 107 and selected from serine and glycine;

(viii) the thirteenth substitution is at position 123 and selected from asparagine, glutamine, and serine;

(ix) the thirteenth substitution is at position 126 and selected from serine and glutamic acid;

(x) the thirteenth substitution is at position 127 and selected from glycine and threonine;

(xi) the thirteenth substitution is at position 129 and selected from cysteine, lysine, and valine;

(xii) the thirteenth substitution is at position 137 and selected from glycine, alanine, aspartic acid, histidine, asparagine, serine, and threonine;

(xiii) the thirteenth substitution is at position 159 and is leucine;

(xiv) the thirteenth substitution is at position 176 and selected from leucine, valine, lysine, arginine, and tyrosine, and optionally a fourteenth substitution of valine at position 248;

(xv) the thirteenth substitution is at position 185 and selected from valine and leucine;

(xvi) the thirteenth substitution is at position 190 and selected from glutamine, alanine and proline, and optionally a fifteenth substitution of serine at position 122;

(xvii) the thirteenth substitution is at position 191 and selected from serine, valine, leucine, isoleucine, tyrosine, and lysine;

(xviii) the thirteenth substitution is at position 192 and selected from serine;

(xix) the thirteenth substitution is at position 195 and selected from isoleucine, and valine;

(xx) the thirteenth substitution is at position 212 and is leucine;

(xxi) the thirteenth substitution is at position 268 and selected from lysine, glutamine, histidine, arginine, and leucine;

(xxii) the thirteenth substitution is at position 269 and selected from leucine, glycine, arginine, and tryptophan; and

(xxiii) the thirteenth substitution is at position 272 and is alanine.

39. The modified enzyme according to claim 25 wherein the modified enzyme comprises a sequence selected from the group consisting of SEQ ID No. 15, SEQ ID No. 14, SEQ ID No. 13, SEQ ID No. 16, SEQ ID No. 12, SEQ ID No. 11, SEQ ID No. 10, SEQ ID No. 9, SEQ ID No. 8, SEQ ID No. 7, SEQ ID No. 6, SEQ ID No. 5, SEQ ID No. 4, and SEQ ID No. 20 to 100.

40. The modified enzyme according to any of claims 25 to 39 further comprising a polyhistidine-tag.

41. A host cell comprising a plurality of nucleic acid sequences to encode enzymes to allow the host cell to convert glucose or glycerol to psi-ionone and a first nucleic acid sequence encoding the modified enzyme according to any of claims 25 to 40.

42. The host cell according to claim 41 wherein the enzymes include at least one of the following: HMG-CoA synthase (HmgS), Acetoacetyl-CoA thiolase (AtoB), truncated HMG-CoA reductase (tHmgR) or HMG-CoA reductase, mevalonate kinase (MevK), phosphomevalonate kinase (PMK), mevalonate pyrophosphate decarboxylase (PMD), IPP isomerase (Idi), GGPP synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI), FPP synthase (IspA), and modified OfCCD1 enzyme or OfCCD1 enzyme.

43. The host cell according to claim 41 or claim 42 wherein at least one of the following conditions is fulfilled:

(i) further comprising a second nucleic acid sequence to encode SAM cycle enzymes;

(ii) a third nucleic acid sequence encoding a transcription regulator metJ is absent;

(iii) comprising at least one T7 promoter sequence;

(iv) the host cell is *Escherichia coli*.

44. A method of methylating a hydroxyl group, the method comprising providing an enzyme selected from the group consisting of the modified enzyme according to any one of claims 25 to 40 (preferably SEQ ID No. 8), SEQ ID No. 1, and SEQ ID

No. 3, or the host cell according to any one of claims 41 to 43; and contacting the enzyme or the host cell and a hydroxyl group under suitable conditions to methylate the hydroxyl group.

45. The method according to claim 44 wherein the hydroxyl group is an allylic hydroxyl group.