

WHAT IS CLAIMED:

1. A genetically modified host cell comprising a heterologous nucleic acid encoding a UDP-glycosyltransferase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 11.
2. The genetically modified host cell of claim 1 that is capable of converting RebA to RebD at an efficiency of greater than 90%, 95%, 96%, or 97%.
3. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises an amino acid sequence having the sequence of SEQ ID NO:1 or SEQ ID NO:11.
4. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase is capable of beta 1,2 glycosylation of the C2' position of the 19-O glucose of a steviol glycoside.
5. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase is encoded by a heterologous nucleic acid, wherein the heterologous nucleic acid comprises a nucleotide sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:13.
6. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase is encoded by a heterologous nucleic acid having the sequence of SEQ ID NO:13.
7. The genetically modified host cell of any of the preceding claims, that is capable of converting RebA to RebD at an efficiency of greater than 90%, 95%, 96%, or 97%, and wherein the UDP-glycosyltransferase comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:1 or SEQ ID NO:11.
8. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase is a polypeptide having a functional domain

with at least 80%, 85%, 90%, or 95% sequence identity to a functional domain of SEQ ID NO:1 or SEQ ID NO: 11.

9. The genetically modified host cell of claim 8, wherein the functional domain is a sugar acceptor domain.

10. The genetically modified host cell of claim 9, wherein the sugar acceptor domain is located at the N-terminus of the UDP-glycosyltransferase.

11. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises a sugar acceptor domain of SEQ ID NO:1 or SEQ ID NO:11.

12. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises a sugar acceptor domain, wherein the amino acid sequence of the sugar acceptor domain has at least 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of the sugar acceptor domain of SEQ ID NO:1 or SEQ ID NO:11.

13. The genetically modified host cell of claim 12, wherein the UDP-glycosyltransferase comprises:

(a) the loop1 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, the sequence of SEQ ID NO:28, or a variant loop1 amino acid sequence, at a location of the UDP-glycosyltransferase that corresponds to the loop1 location of SEQ ID NO:1 or SEQ ID NO:11, respectively;

(b) the loop2 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, or a variant loop2 amino acid sequence, at a location of the UDP-glycosyltransferase that corresponds to the loop2 location of SEQ ID NO:1 or SEQ ID NO:11, respectively;

(c) the loop3_1 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, or a variant loop3_1 amino acid sequence, at a location of the UDP-glycosyltransferase that corresponds to the loop 3_1 location of SEQ ID NO:1 or SEQ ID NO:11, respectively;

(d) the loop3_2 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, or a variant loop3_2 amino acid sequence, at a location of the UDP-glycosyltransferase that corresponds to the loop3_2 location of SEQ ID NO:1 or SEQ ID NO:11, respectively;

(e) the loop4_1 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, or a variant loop4_1 amino acid sequence, at a location that corresponds to the loop4_1 location of SEQ ID NO:1 or SEQ ID NO:11, respectively;

(f) the loop4_2 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11, at a location that corresponds to the loop4_2 location of SEQ ID NO:1 or SEQ ID NO:11, respectively; or

(g) any combination of (a) through (f).

14. The genetically modified host cell of claim 13, wherein any of the variant loop1 amino acid sequence, the variant loop2 amino acid sequence, the variant loop3_1 amino acid sequence, the variant loop 3_2 amino acid sequence, and the variant loop4_1 amino acid sequence are obtained from their respective, corresponding loop locations of another UDP-glycosyltransferase different from SEQ ID NO:1 or SEQ ID NO:11, wherein another UDP-glycosyltransferase comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:1 or SEQ ID NO: 11.

15. The genetically modified host cell of 14, wherein another UDP-glycosyltransferase is capable of beta 1,2 glycosylation of the C'2 position of the 19-O-glucose of a steviol glycoside.

16. The genetically modified host cell of any of claim 15, wherein another UDP-glycosyltransferase is Os_UGT_91C1.

17. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises:

(a) a sugar acceptor domain of the another UDP-glycosyltransferase comprising an amino acid sequence having at least 61%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to the sugar acceptor domain of SEQ ID NO:1 or SEQ ID NO:11; and

(b) the loop4_1 amino acid sequence of SEQ ID NO:1 or SEQ ID NO: 11 at a location of another UDP-glycosyltransferase that corresponds to the loop4_1 location of SEQ ID NO:1 or SEQ ID NO:11, respectively.

18. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises the sequence of SEQ ID NO:22 as the sugar acceptor domain.

19. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:1 or SEQ ID NO:11 and has the loop4_1 amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11 at a location that corresponds to the loop4_1 location of SEQ ID NO:1 or SEQ ID NO:11, respectively.

20. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises the sequence of SEQ ID NO:25.

21. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises one or more of the following amino acid residues:

(a) valine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 11 of SEQ ID NO:11;

(b) isoleucine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 12 of SEQ ID NO:11;

(c) proline at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 55 of SEQ ID NO:11;

(d) glutamic acid at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 90 of SEQ ID NO:11;

(e) serine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 203 of SEQ ID NO:11;

(f) glutamic acid at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 223 of SEQ ID NO:11; or

(g) valine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 413 of SEQ ID NO:11,

wherein the amino acid positions of the UDP-glycosyltransferase that correspond to the amino acid positions of SEQ ID NO:11 are determined by sequence alignment.

22. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises an amino acid sequence having SEQ ID NO:24.

23. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises a sugar donor domain, wherein the sugar donor domain comprising an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of the sugar donor domain of SEQ ID NO:1 or SEQ ID NO: 11.

24. The genetically modified host cell of any of the preceding claims, wherein the sugar donor domain is located at the C-terminus of the UDP-glycosyltransferase.

25. The genetically modified host cell of any of the preceding claims, wherein the UDP-glycosyltransferase comprises an amino acid sequence having at least 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1 or SEQ ID NO:11.

26. A genetically modified host cell comprising a heterologous nucleic acid encoding a UDP-glycosyltransferase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:2.

27. The genetically modified host cell of claim 26 that is capable of converting RebA to RebD at an efficiency of greater than 50%.

28. The genetically modified host cell of claim 26 or 27, wherein the UDP-glycosyltransferase is a polypeptide having the sequence of SEQ ID NO:2.

29. The genetically modified host cell of any one of claims 26 to 28, wherein the UDP-glycosyltransferase is a polypeptide comprising a functional domain with at least 80%, 85%, 90%, or 95% sequence identity to a functional domain of SEQ ID NO:2.

30. A genetically modified host cell comprising a heterologous nucleic acid encoding a UDP-glycosyltransferase comprising an amino acid sequence having at least 80%, 85%, 90%, or 95% sequence identity to SEQ ID NO:5.

31. The genetically modified host cell of claim 30 that is capable of converting RebA to RebD at an efficiency of greater than 40%

32. The genetically modified host cell of claim 30 or 31, wherein the UDP-glycosyltransferase comprises an amino acid sequence having the sequence of SEQ ID NO:5.

33. The genetically modified host cell of any of claims 30 to 32, wherein the UDP-glycosyltransferase comprises a polypeptide having a functional domain with at least 80%, 85%, 90%, or 95% sequence identity to a functional domain of SEQ ID NO:5.

34. A genetically modified host cell comprising a heterologous nucleic acid encoding a UDP-glycosyltransferase comprising an amino acid sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:6.

35. The genetically modified host cell of claim 34 that is capable of converting RebA to RebD at an efficiency of greater than 85% or 90%.

36. The genetically modified host cell of claim 34 or 35, wherein the UDP-glycosyltransferase is a polypeptide having the sequence of SEQ ID NO:6.

37. The genetically modified host cell of any one of claims 34 to 36, wherein the UDP-glycosyltransferase comprises a polypeptide having a functional domain with at least 80%, 85%, 90%, or 95% sequence identity to a functional domain of SEQ ID NO:6.

38. The genetically modified host cell of any of claims 29, 33, and 37, wherein the functional domain is a sugar acceptor domain.

39. The genetically modified host cell of any of the preceding claims that is capable of producing RebD.

40. The genetically modified host cell of any of the preceding claims that is capable of producing RebM.

41. The genetically modified host cell of any of the preceding claims that is capable of producing at RebM and RebM2 at a ratio of at least 10:1, 100:1, or 1000:1.

42. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell produces an undetectable level of RebM2.

43. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell is capable of converting stevioside to RebE.

44. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making steviol.

45. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making a steviol glycoside.

46. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making a RebA.

47. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making RebM.

48. The genetically modified host cell of any of the preceding claims, wherein the genetically modified host cell further comprises one or more heterologous nucleic acids encoding one or more enzymes of a pathway for making RebE.

49. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a geranylgeranyl diphosphate synthase.

50. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a copalyl diphosphate synthase.

51. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise an ent-kaurene synthase.

52. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a kaurene oxidase.

53. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a kaurenoic acid hydroxylase.

54. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a cytochrome P450 reductase.

55. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a uridine 5'-diphospho glycosyltransferase.

56. The genetically modified host cell of any of the preceding claims wherein the one or more enzymes of the pathway comprise UGT74G1, UGT76G1, UGT85C2, and UGT91D.

57. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprise a geranylgeranyl diphosphate synthase, a copalyl diphosphate synthase, a ent-kaurene synthase, a kaurene oxidase, a kaurenoic acid hydroxylase, a cytochrome P450 reductase, UGT74G1, UGT76G1, UGT85C2, and UGT91D.

58. The genetically modified host cell of any of the preceding claims, wherein the one or more enzymes of the pathway comprises a bifunctional copalyl diphosphate synthase and kaurene synthase.

59. The genetically modified host cell of any of the preceding claims, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the pathway are under control of a single transcriptional regulator.

60. The genetically modified host cell of any of the preceding claims, wherein the one or more heterologous nucleic acids encoding one or more enzymes of the pathway are under control of multiple heterologous transcriptional regulators.

61. The genetically modified host cell of any one of any of the preceding claims, wherein the cell is selected from the group consisting of a bacterial cell, a fungal cell, an algal cell, an insect cell, and a plant cell.

62. The genetically modified host cell of any of the preceding claims, wherein the cell is a yeast cell.

63. The genetically modified host cell of any of the preceding claims, wherein the yeast is *Saccharomyces cerevisiae*.

64. A method for producing RebD:

- (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebD; and
- (b) recovering said RebD compound from the medium.

65. A method for producing RebM:

- (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and
- (b) recovering said RebM compound from the medium.

66. A method for producing RebD:

- (a) contacting RebA with glucose and a UDP-glycosyltransferase of any of the preceding claims, capable of converting RebA to RebD, under conditions suitable for forming RebD; and

- (b) recovering said RebD compound from the medium.

67. A method of producing RebM:

- (a) contacting, under conditions suitable for forming RebM, a substrate with glucose or a UDP-sugar and:
 - (i) a UDP-glycosyltransferase of any of the preceding claims, capable of converting RebA to RebD; and
 - (ii) a UDP-glycosyltransferase capable of converting RebD to RebM.

68. A method for making RebD comprising converting RebA to RebD using any of the UDP-glycosyltransferase of any of the preceding claims, capable of converting RebA to RebD.

69. A method of making RebM comprising:

- (a) converting RebA to RebD using any of the UDP-glycosyltransferase of any of the preceding claims, capable of converting RebA to RebD; and
- (b) converting RebD to RebM using a UDP-glycosyltransferase capable of converting RebD to RebM.

70. A fermentation composition comprising:

- (a) a genetically modified host cell comprising:
 - (i) a heterologous nucleic acid encoding a UDP-glycosyltransferase of any of the preceding claims, capable of converting RebA to RebD; and
- (b) steviol glycosides produced from the genetically modified host cell.

71. A fermentation composition produced by the genetically modified host cells of any of the preceding claims comprising steviol glycosides, wherein the steviol glycosides comprise RebA, RebD and RebM at a RebA:RebD:RebM ratio of at least 1:7:50.

72. The fermentation composition of claim 71, wherein the steviol glycosides comprise RebA, RebD and RebM at a RebA:RebD:RebM ratio of at least 1:7:50 to 1:100:1000.

73. The fermentation composition of claim 71, wherein the steviol glycosides comprise RebA, RebD and RebM at a RebA:RebD:RebM ratio of at least 1:7:50 to 1:200:2000.

74. A fermentation composition produced by the genetically modified host cells of any of the preceding claims comprising steviol glycosides, wherein the steviol glycosides comprise RebD and RebM at a RebD:RebM ratio of at least 7:50.

75. The fermentation composition of claim 74, wherein the steviol glycosides comprise RebD and RebM at a RebD:RebM ratio of at least 7:50 to 7:100.

76. The fermentation composition of claim 74, wherein the steviol glycosides comprise RebD and RebM at a RebD:RebM ratio of at least 7:50 to 7:200.

77. A fermentation composition produced by the genetically modified host cells of any of the preceding claims comprising steviol glycosides, wherein the steviol glycosides comprise RebA and RebM at a RebA:RebM ratio of at least 1:50.

78. The fermentation composition of claim 77, wherein the steviol glycosides comprise RebA and RebM at a RebA:RebM ratio of at least 1:50 to 1:1000.

79. The fermentation composition of claim 77, wherein the steviol glycosides comprise RebA and RebM at a RebA:RebM ratio of at least 1:50 to 1:2000.

80. A non-naturally occurring UDP-glycosyltransferase having at least 80%, 85%, 90%, 95% sequence identity to SEQ ID NO:1 or SEQ ID NO:11.

81. The non-naturally occurring UDP-glycosyltransferase that is capable of converting RebA to RebD at an efficiency of greater than 90%, 95%, 96%, or 97% *in vivo*.

82. A non-naturally occurring UDP-glycosyltransferase, wherein the UDP-glycosyltransferase:

(a) comprises the loop4_1 amino acid sequence having SEQ ID NO:22 at a location of the UDP-glycosyltransferase that corresponds to the loop4_1 location of SEQ ID NO:1 or SEQ ID NO:11; and

(b) is capable of beta 1,2 glycosylation of the C2' position of the 19-O-glucose of a steviol glycoside.

83. The non-naturally occurring UDP-glycosyltransferase of any of claims 80 to 82, wherein the UDP-glycosyltransferase comprises one or more of the following amino acid residues:

- (a) valine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 11 of SEQ ID NO:11;
 - (b) isoleucine at an amino acid position of UDP-glycosyltransferase that corresponds to amino acid position 12 of SEQ ID NO:11;
 - (c) proline at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 55 of SEQ ID NO:11;
 - (d) glutamic acid at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 90 of SEQ ID NO:11;
 - (e) serine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 203 of SEQ ID NO:11;
 - (f) glutamic acid at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 223 of SEQ ID NO:11; or
 - (g) valine at an amino acid position of the UDP-glycosyltransferase that corresponds to amino acid position 413 of SEQ ID NO:11,
- wherein the amino acid positions of the UDP-glycosyltransferase that correspond to the amino acid positions of SEQ ID NO:11 are determined by sequence alignment.

84. A non-naturally occurring nucleic acid encoding the non-naturally occurring UDP-glycosyltransferase of any of claims 80 to 82.

85. A sweetener comprising RebM made by the process of

- (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and
- (b) recovering said RebM compound from the medium.

86. A sweetener composition comprising at least one other substance and RebM made by the process of

- (a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and
- (b) recovering said RebM compound from the medium.

87. A sweetened composition comprising RebM made by the process of

(a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and

(b) recovering said RebM compound from the medium.

88. A method of making a sweetener composition comprising combining at least one other substance with RebM made by the process of

(a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and

(b) recovering said RebM compound from the medium.

89. A method of making a sweetened composition comprising combining at least one sweetenable composition with RebM made by the process of

(a) culturing a population of the genetically modified host cells of any of the preceding claims in a medium with a carbon source under conditions suitable for making RebM; and

(b) recovering said RebM compound from the medium.