CLAIMS

1. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that is at least about 75% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

2. The enzyme of claim 1, wherein the amino acid sequence is at least about 80% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

3. The enzyme of claim 1, wherein the amino acid sequence is at least about 85% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

4. The enzyme of claim 1, wherein the amino acid sequence is at least about 90% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

5. The enzyme of claim 1, wherein the amino acid sequence is at least about 95% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

6. The enzyme of claim 1, wherein the amino acid sequence is at least about 98% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

7. The enzyme of claim 1, wherein the amino acid sequence is the amino acid of SEQ ID NO: 5 or SEQ ID NO: 6.

8. The enzyme of claim 1, wherein the amino acid sequence has from 1 to 20 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence SEQ ID NO: 5 or SEQ ID NO: 6.

9. The enzyme of claim 8, wherein the amino acid sequence has from 1 to 10 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

10. The enzyme of claim 8, wherein the amino acid sequence has from 1 to 5 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

11. The enzyme of any one of claims 8 to 10, wherein the enzyme comprises an amino acid substitution at positions corresponding to one or more of positions 29, 200, 357, and 414 of SEQ ID NO: 1.

12. The enzyme of any one of claims 1 to 11, comprising one or more of:
a glycine (G) at the position corresponding to position 54 of SEQ ID NO: 5;
a leucine (L) at the position corresponding to position 111 of SEQ ID NO: 5; and
a methionine (M) at the position corresponding to position 183 of SEQ ID NO: 5.

13. The enzyme of claim 12, wherein the enzyme has Gly at the position corresponding to position 54 of SEQ ID NO: 5, Leu at the position corresponding to position 111 of the SEQ ID NO: 5, and a Met at the position corresponding to position 183 of SEQ ID NO: 5.

14. The enzyme of any one of claims 1 to 13, wherein the enzyme comprises an insertion of from about 6 to about 12 amino acids after the position corresponding to position 155 of SEQ ID NO: 5.

15. The enzyme of claim 14, wherein the insertion is a flexible and hydrophilic sequence that is predominately Glycine and Serine residues, and is optionally GSGGSG (SEQ ID NO: 7) or GSGGSGGSG (SEQ ID NO: 8).

16. The enzyme of any one of claims 1 to 15, wherein the enzyme comprises a deletion of one or more amino acids corresponding to amino acids E225 to T232 with respect to the amino acid sequence of SEQ ID NO: 6.

17. The enzyme of claim 16, wherein the enzyme comprises a deletion of the amino acids corresponding to amino acids E225 to T232 of SEQ ID NO: 6.

18. The enzyme of any one of claims 1 to 17, wherein the enzyme comprises an amino acid substitution at one or more positions corresponding to position 72, position 305, position 345, and position 428 of SEQ ID NO: 6.

19. The enzyme of claim 18, wherein the enzyme comprises one or more amino acid substitutions selected from: a glutamine (Q) at the position corresponding to position 72 of SEQ ID NO: 6; a cysteine (C) at the position corresponding to position 305 of SEQ ID NO: 6; a phenylalanine (F) at the position corresponding to position 345 of SEQ ID NO: 6; and an isoleucine (I) at the position corresponding to position 428 of SEQ ID NO: 6.

20. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that is at least about 60% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6, wherein the UGT enzyme comprises:

a glycine (G) at the position corresponding to position 54 of SEQ ID NO: 5;

a leucine (L) at the position corresponding to position 111 of SEQ ID NO: 5; and

a methionine (M) at the position corresponding to position 183 of SEQ ID NO: 5.

21. The enzyme of claim 20, wherein the amino acid sequence is at least about 70% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

22. The enzyme of claim 20, wherein the amino acid sequence is at least about 80% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

23. The enzyme of claim 20, wherein the amino acid sequence is at least about 90% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

24. The enzyme of claim 20, wherein the amino acid sequence is at least about 95% to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

25. The enzyme of any one of claims 20 to 24, wherein the 1-3 UGT enzyme comprises an insertion of from about 5 to about 15 amino acids after the position corresponding to positions 155 of SEQ ID NO: 5.

26. The enzyme of claim 25, wherein the insertion is a flexible and hydrophilic sequence that is predominately Glycine and Serine residues, and is optionally GSGGSG (SEQ ID NO: 7) and GSGGSGGSG (SEQ ID NO: 8).

27. The enzyme of any one of claims 20 to 26, wherein the enzyme comprises a deletion of one or more amino acids corresponding to amino acids E225 to T232 with respect to the amino acid sequence of SEQ ID NO: 6.

28. The enzyme of claim 27, wherein the enzyme comprises a deletion of the amino acids corresponding to amino acids E225 to T232 of SEQ ID NO: 6.

29. The enzyme of any one of claims 20 to 28, wherein the enzyme comprises an amino acid substitution at one or more positions corresponding to position 72, position 305, position 345, and position 428 of SEQ ID NO: 6.

30. The enzyme of claim 29, wherein the enzyme comprises one or more amino acid substitutions selected from: a glutamine (Q) at the position corresponding to position 72 of SEQ ID NO: 6; a cysteine (C) at the position corresponding to position 305 of SEQ ID NO: 6; a phenylalanine (F) at the position corresponding to position 345 of SEQ ID NO: 6; and an isoleucine (I) at the position corresponding to position 428 of SEQ ID NO: 6.

31. The enzyme of any one of claims 20 to 30 wherein the amino acid sequence is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 98% identical to the amino acid sequence of SEQ ID NO: 9.

32. The enzyme of any one of claims 20-31, wherein the amino acid sequence has from 1 to 20 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence SEQ ID NO: 9.

33. The enzyme of any one of claims 20-31, wherein the amino acid sequence has from 1 to 10 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence of SEQ ID NO: 9.

34. The enzyme of any one of claims 20-31, wherein the amino acid sequence has from 1 to 5 amino acid modifications independently selected from substitutions, deletions, and insertions, with respect to the amino acid sequence of SEQ ID NO: 9.

35. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that has at least about 75% sequence identity to the amino acid sequence of SEQ ID NO:1, and comprising one or more modifications with respect to SEQ ID NO:1 selected from:

a deletion of one or more amino acids corresponding to E74 to T81,

a substitution at the position corresponding to position 357, which is optionally glycine,

a substitution at the position corresponding to position 414, and which is optionally leucine,

a substitution at the position corresponding to position 29, and which is optionally methionine,

a substitution at the position corresponding to position 402, which is optionally glutamine,

a substitution at the position corresponding to position 154, which is optionally cysteine,

a substitution at the position corresponding to position 194, and which is optionally phenylalanine,

a substitution at the position corresponding to position 277, and which is optionally isoleucine,

a substitution at the position corresponding to position 208, and which is optionally glutamine,

a substitution at the position corresponding to position 140, and which is optionally proline, and

a substitution at the position corresponding to position 259, and which is optionally glutamic acid.

36. The UGT enzyme of claim 35, wherein the UGT enzyme comprises a deletion of at least two or at least three amino acids corresponding to E74 to T81.

37. The UGT enzyme of claim 35, wherein the UGT enzyme comprises a deletion of at least four or at least five amino acids corresponding to E74 to T81.

38. The UGT enzyme of claim 35, wherein the UGT enzyme comprises a deletion of at least six or at least seven amino acids corresponding to E74 to T81.

39. The UGT enzyme of claim 35, wherein the UGT enzyme comprises a deletion of the amino acids corresponding to E74 to T81.

40. The UGT enzyme of any one of claims 35 to 39, wherein the enzyme further comprises a substitution of alanine at the position corresponding to position 200 of SEQ ID NO: 1.

41. The UGT enzyme of any one of claims 35 to 40, wherein the enzyme has at least 2, 3, or 4 of: 357G, 414L, 29M, 402Q, 154C, 194F, 277I, 208Q, 140P, and 259E, each numbered according to SEQ ID NO:1

42. The UGT enzyme of any one of claims 35 to 41, wherein the enzyme is at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 98% identical to the amino acid sequence of SEQ ID NO:1.

43. The UGT enzyme of claim 42, wherein the enzyme has from 1 to 20, or from 1 to 10 amino acid modifications independently selected from amino acid substitutions, deletions, and insertions with respect to SEQ ID NO:1.

44. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme that is a circular permutant of SrUGT76G1, and optionally having from 1 to 20 or from 1 to 15, or from 1 to 10 amino acid modifications independently selected from amino acid substitutions, deletions, and insertions with respect to the corresponding position of SEQ ID NO:1, with the proviso that the circular permutant one or more modifications with respect to SEQ ID NO:1 selected from:

a deletion of one or more amino acids corresponding to E74 to T81,

a substitution at the position corresponding to position 357, which is optionally glycine,

a substitution at the position corresponding to position 414, and which is optionally leucine,

a substitution at the position corresponding to position 29, and which is optionally methionine,

a substitution at the position corresponding to position 402, which is optionally glutamine,

a substitution at the position corresponding to position 154, which is optionally cysteine,

a substitution at the position corresponding to position 194, and which is optionally phenylalanine,

a substitution at the position corresponding to position 277, and which is optionally isoleucine,

a substitution at the position corresponding to position 208, and which is optionally glutamine,

a substitution at the position corresponding to position 140, and which is optionally proline, and

a substitution at the position corresponding to position 259, and which is optionally glutamic acid.

45. A polynucleotide encoding the enzyme of any one of claims 1 to 44.

46. A host cell comprising the polynucleotide of claim 45.

47. The host cell of claim 46, wherein the cell is a microorganism.

48. The host cell of claim 47, wherein the microorganism is a bacterium.

49. The host cell of claim 48, wherein the bacterium is *E. coli*.

50. The host cell of claim 49, wherein the host cell comprises a deletion or inactivation of ushA and galETKM, a deletion or inactivation of pgi, and an overexpression or increased activity of pgm and galU.

51. The host cell of claim 47, wherein the microorganism is a yeast.

52. The host cell of claim 46, wherein the cell is a plant.

53. The host cell of claim 52, wherein the plant is a stevia plant.

54. The host cell of any one of claims 46 to 53, wherein the host cell expresses one or more additional UGT enzymes selected from C-13 UGT enzyme, a C-19 UGT enzyme, and a 1-2' UGT enzyme.

55. The host cell of claim 54, wherein the host cell further expresses a heterologous C-13 UGT enzyme, a heterologous C-19 UGT enzyme, and a heterologous 1-2 UGT enzyme.

56. The host cell of any one of claims 47 to 55, wherein the host cell produces steviol by expression of endogenous and/or heterologous enzymes for biosynthesis of steviol.

57. A method for transferring a monosaccharide group to a substrate comprising contacting a NDP-sugar and the substrate with the UGT enzyme of any one of claims 1 to 44, or the host cell or lysate thereof of any one of claims 46 to 56.

58. The method of claim 57, wherein the substrate is a terpenoid.

59. The method of claim 58, wherein the terpenoid is a diterpenoid.

60. The method of claim 59, wherein the diterpenoid is steviol and/or a steviol glycoside.

61. The method of claim 60, wherein the steviol glycoside is stevioside and/or RebD.

62. The method of claim 57, where the substrate is a plant extract, which is optionally a stevia leaf extract.

63. The method of claim 58, wherein the terpenoid is a monoterpenoid, sesquiterpenoid, or triterpenoid.

64. The method of claim 63, wherein the terpenoid is a triterpenoid, which is optionally mogrol or a mogroside.

65. The method of any one of claims 57 to 64, wherein the NDP-sugar is UDP-glucose.

66. The method of claim 65, wherein the reaction takes place in a host cell with endogenous UDP-glucose.

67. A method for transferring a glycosyl group to a substrate comprising growing the host cell of any one of claims 46 to 56 in the presence of the substrate.

68. The method of claim 67, wherein the substrate is synthesized by the host cell.

69. The method of claim 67 or 68, wherein the substrate is a terpenoid.

70. The method of claim 69, wherein the terpenoid is a diterpenoid.

71. The method of claim 70, wherein the diterpenoid is steviol and/or a steviol glycoside.

72. The method of claim 71, wherein the steviol glycoside is stevioside and/or RebD.

73. The method of claim 69, wherein the terpenoid is a triterpenoid, which is optionally mogrol or a mogroside.

CLAIMS

1. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6, wherein:

the enzyme has an insertion with respect to SEQ ID NO: 1 of a flexible and hydrophilic sequence of 6 to 12 amino acids that is predominately Glycine and Serine residues after the position corresponding to position 155 of SEQ ID NO: 5; and

the enzyme retains one or more of:

a glycine (G) at the position corresponding to position 54 of SEQ ID NO: 5;

a leucine (L) at the position corresponding to position 111 of SEQ ID NO: 5; and

a methionine (M) at the position corresponding to position 183 of SEQ ID NO: 5.

2. The enzyme of claim 1, wherein the amino acid sequence is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

3. The enzyme of claim 1, wherein the enzyme has Gly at the position corresponding to position 54 of SEQ ID NO: 5, Leu at the position corresponding to position 111 of the SEQ ID NO: 5, and a Met at the position corresponding to position 183 of SEQ ID NO: 5.

4. The enzyme of claim 1, wherein the insertion is GSGGSG (SEQ ID NO: 7) or GSGGSGGSG (SEQ ID NO: 8).

5. The enzyme of claim 1, wherein the enzyme comprises a deletion of at least three amino acids corresponding to amino acids E225 to T232 with respect to the amino acid sequence of SEQ ID NO: 6.

6. The enzyme of claim 5, wherein the enzyme comprises a deletion of the amino acids corresponding to amino acids E225 to T232 of SEQ ID NO: 6.

7. The enzyme of claim 1, wherein the enzyme comprises an amino acid substitution at one or more positions corresponding to position 72, position 305, position 345, and position 428 of SEQ ID NO: 6.

8. The enzyme of claim 7, wherein the enzyme comprises one or more amino acid substitutions selected from: a glutamine (Q) at the position corresponding to position 72 of SEQ ID NO: 6; a cysteine (C) at the position corresponding to position 305 of SEQ ID NO: 6; a phenylalanine (F) at the position corresponding to position 345 of SEQ ID NO: 6; and an isoleucine (I) at the position corresponding to position 428 of SEQ ID NO: 6.

9. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6, wherein the UGT enzyme comprises:

a glycine (G) at the position corresponding to position 54 of SEQ ID NO: 5;

a leucine (L) at the position corresponding to position 111 of SEQ ID NO: 5; and

a methionine (M) at the position corresponding to position 183 of SEQ ID NO: 5.

10. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that has at least 85% sequence identity to the amino acid sequence of SEQ ID NO:1, and comprising a deletion of amino acids corresponding to E74 to T81 of SEQ ID NO: 1.

11. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme that is a circular permutant of SrUGT76G1 (SEQ ID NO: 1), and having from 1 to 20 amino acid modifications independently selected from amino acid substitutions, deletions, and insertions with respect to the corresponding position of SEQ ID NO:1, with the proviso that the circular permutant has a deletion of amino acids corresponding to amino acids E74 to T81 of SEQ ID NO: 1.

12. The enzyme of claim 5, wherein the enzyme comprises a deletion of at least five amino acids corresponding to amino acids E74 to T81 with respect to the amino acid sequence of SEQ ID NO: 6.

13. The enzyme of claim 7, wherein the enzyme comprises a substitution of glutamine (Q) or asparagine (N) at the position corresponding to position 72 of SEQ ID NO: 6.

14. The enzyme of claim 7, wherein the enzyme comprises a neutral hydrophilic amino acid selected from cysteine (C), Serine (S), or Threonine (T) at the position corresponding to position 305 of SEQ ID NO: 6.

15. The enzyme of claim 7, wherein the enzyme comprises a substitution of phenylalanine (F) or Tryptophan (W) at the position corresponding to position 345 of SEQ ID NO: 6.

16. The enzyme of claim 7, wherein the enzyme comprises a substitution of isoleucine (I), valine (V), or alanine (A) at the position corresponding to position 428 of SEQ ID NO: 6.

17. The enzyme of claim 7, wherein the enzyme comprises 2, 3, or 4 of the following substitutions with respect to SEQ ID NO: 6: S72Q, A305C, Y345F, and L428I.

18. A uridine diphosphate-dependent glycosyltransferase (UGT) enzyme comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6, and comprising a deletion of at least three amino acids corresponding to amino acids E225 to T232 with respect to SEQ ID NO: 6.

19. The UGT enzyme of claim 18, wherein the enzyme comprises a deletion of at least five amino acids corresponding to amino acids E225 to T232 with respect to SEQ ID NO: 6.

20. The UGT enzyme of claim 18, wherein the enzyme comprises a deletion of the amino acids corresponding to amino acids E225 to T232 with respect to SEQ ID NO: 6.

21. The UGT enzyme of claim 18, wherein the enzyme is at least 90% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

22. The UGT enzyme of claim 18, wherein the enzyme is at least 95% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

23. A polynucleotide encoding the enzyme of any one of claims 1, 9, 10, 11 or 18.

24. An isolated recombinant microorganism comprising the polynucleotide of claim 23.

25. A method for transferring a monosaccharide group to a substrate comprising contacting a NDP-sugar and the substrate with the isolated recombinant microorganism of claim 24.

26. A method for transferring a glycosyl group to a substrate comprising growing the isolated recombinant microorganism of claim 24 in the presence of the substrate.