additional amino acids at the N-terminus, relative to the six sequences identified by pyrosequencing. To re-test UGT91D1 family members for activity, UGT91D1 sequences were re-isolated by PCR amplification of *Stevia* leaf cDNA. The resulting PCR products were cloned into a plasmid vector and enzymatic activity for each product was measured as described above by: GST-tagged expression in *E. coli*, coupled *in vitro* transcription-translation, and/or *in vivo* expression in yeast. Steviol 1,2-glucosylation activity was detected from one clone by all three methods. This clone was designated UGT91D2e. The amino acid sequence of UGT91D2e is set forth in SEQ ID NO:5. In contrast, no 1,2-glucosylation activity was detected from a clone having the same sequence as described by Accession No. AY345980 (Protein Accession number AAR06918), but lacking the 12 amino acids of the amino terminus.

Example 11 – Analysis of UGT91D2e Sequences

Sequence variants of UGT91D2e

As evidenced in FIG. ~~19B~~8, a small number of amino acid modifications exist between the active (91D2e) variants and the closest inactive homologs (91D1). The 91D1 genes cloned by Ma et al., Shi Yan Sheng Wu Xue Bao. 2003 36(2):123-9 (Protein Accession number AAM53963, GI:21435782) and Brandle et al., *supra* (Protein Accession number AAR06918, GI:37993665) did not exhibit the 1,2-glycosylating activity required for RebA biosynthesis. To ascertain which amino acids are required for activity, 21 single site-directed mutants were created such that the amino acid in UGT91D2e (SEQ ID NO:5) was changed to the corresponding amino acid in an inactive homolog. See Table 12. In addition, a site-directed mutation was made such that position 364 (S→P) also was changed. The mutants were made using the QuikChange® II Site-Directed Mutagenesis kit according to manufacturer's protocols (Agilent Technologies, Santa Clara, CA), and the pGEX-4TI vectors were transformed into a XJb Autolysis *E. coli* strain (ZymoResearch, Orange, CA). A mutant was not made to change residue 162 from a glycine to an aspartic acid.

In order to assess the activity of the mutant enzymes, a substrate-feeding experiment was performed *in vitro* using protein produced in *E. coli*. Initially, *E. coli* cells were grown overnight at 30°C, followed by induction with 3 mM arabinose and

WHAT IS CLAIMED IS:

1. A recombinant host comprising:
    a) a recombinant gene encoding a UGT91D2 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5; and one or more of:
    (b) a recombinant gene encoding a UGT85C polypeptide, comprising a UGT85C polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3; and/or
    (c) a recombinant gene encoding a UGT76G1 polypeptide, comprising a UGT76G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:7; and/or
    (d) a recombinant gene encoding a UGT74G1 polypeptide, comprising a UGT74G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1.

2. The recombinant host of claim 1, wherein the UGT85C polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3 comprises one or more amino acid substitutions at residues 9, 10, 13, 15, 21, 27, 60, 65, 71, 87, 91, 220, 243, 270, 289, 298, 334, 336, 350, 368, 389, 394, 397, 418, 420, 440, 441, 444, and 471 of SEQ ID NO:3.

3. The recombinant host of claim 1 or claim 2, wherein the UGT76G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:7 comprises one or more amino acid substitutions at residues 29, 74, 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, 266, 273, 274, 284, 285, 291, 330, 331, and 346 of SEQ ID NO:7.

4. The recombinant host of any one of claims 1 to 3, wherein said UGT91D2 polypeptide has at least 95% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

5. The recombinant host of any one of claims 1 to 4, wherein said UGT91D2 polypeptide comprises:

(a) at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5;

(b) an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5;

(c) an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 of SEQ ID NO:5;

(d) a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, a threonine at residue 148, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 221, an alanine at residue 286, an asparagine at residue 427, or an alanine at residue 438 of SEQ ID NO:5;

(e) the amino acid sequence of SEQ ID NO:95;

(f) the amino acid sequence of SEQ ID NO:5; or

(g) a UGT91D2e or a UGT91D2m polypeptide.

6. The recombinant host of any one of claims 1 to 5, said host further comprising one or more of the following:

(a) a gene encoding a geranylgeranyl diphosphate synthase;

(b) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;

(c) a gene encoding a kaurene oxidase;

(d) a gene encoding a steviol synthetase;

(e) a gene encoding a truncated HMG-CoA;

(f) a gene encoding a CPR;

(g) a gene encoding a rhamnose synthetase;

(h) a gene encoding a UDP-glucose dehydrogenase; and

(i) a gene encoding a UDP-glucuronic acid decarboxylase.

7. The recombinant host of claim 6, wherein at least one of the genes of (a), (b), (c), (d), (e), (f), (g), (h) or (i) is a recombinant gene.

8. The recombinant host of claim 7, wherein each of the genes of (a), (b), (c) and (d) is a recombinant gene.

9. The recombinant host of any one of claims 1 to 8, wherein the host is a microorganism, comprising a Saccharomycete such as *Saccharomyces cerevisiae*, *Escherichia coli*, or a plant or plant cell, comprising a *Stevia* such as a *Stevia rebaudiana*, *Physcomitrella patens*, or tobacco plant.

10. The recombinant host according to any one of claims 1 to 9, the host further comprising:

(a) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;

(b) a gene encoding a kaurene oxidase;

(c) a gene encoding a steviol synthetase; or

(d) a gene encoding a geranylgeranyl diphosphate synthase,

wherein at least one of said genes is a recombinant gene;

wherein the host produces at least one steviol glycoside such as rebaudioside A, rebaudioside D or rebaudioside E when cultured under conditions in which said genes are expressed,

wherein the host further comprises a gene encoding a rhamnose synthetase and produces at least one steviol glycoside such as rebaudioside C or dulcoside A when cultured under conditions in which said genes are expressed; or

wherein the host further comprises a gene encoding a UDP-glucose dehydrogenase and a gene encoding a UDP-glucuronic acid decarboxylase and produces at least one steviol glycoside such as rebaudioside F when cultured under conditions in which said genes are expressed.

11. The recombinant host according to any one of claims 1 to 10, further comprising exogenous nucleic acids including:

(a) a recombinant gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:121-128;

(b) a recombinant gene encoding an copalyl diphosphate synthase (CDPS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:129-131;

(c) a recombinant gene encoding a kaurene synthase (KS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:132-135;

(d) a recombinant gene encoding a kaurene oxidase (KO) polypeptide, comprising KO polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:138-141;

(e) a recombinant gene encoding a steviol synthase polypeptide (KAH), comprising a steviol synthase polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:142-146;

(f) a recombinant gene encoding a cytochrome P450 reductase (CPR) polypeptide, comprising a CPR polypeptide having at least 90% sequence identity to any one of the amino acid sequence set forth in SEQ ID NOs: 147-149;

wherein the recombinant host produces a steviol or a steviol glycoside.

12. The recombinant host of any one of claims 1 to 10, wherein the steviol or the steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, and dulcoside A.

13. The recombinant host of any one of claims 1 to 12 for the production of a steviol or a steviol glycoside, wherein one or more of the genes encoding a polypeptide comprises exogenous nucleic acids.

14. An isolated nucleic acid:

(a) encoding a polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5, comprising:

(i) a polypeptide comprising an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 153, 156, 195, 196, 199, 206, 207, 211, 221, 286, 343, 427, and 438 of SEQ ID NO:5; or

(i) a polypeptide comprising an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 of SEQ ID NO:5; or

(iii) a polypeptide comprising a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, a threonine at residue 148, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 221, an alanine at residue 286, an asparagine at residue 427, or an alanine at residue 438 of SEQ ID NO:5; or

(iv) a polypeptide having at least 90% sequence identity to the amino acid sequence of UGT91D2m of SEQ ID NO:10, excluding the amino acid sequence of UGT91D2m of SEQ ID NO:10.

15. An isolated polypeptide having an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:5; or an isolated polypeptide having at least 90% sequence identity to the amino acid sequence of UGT91D2m of SEQ ID NO:10, excluding the amino acid sequence of UGT91Dm of SEQ ID NO:10.

16. A steviol glycoside composition produced by the recombinant host of any one of claims 1-13, said composition comprising:

(a) greater than 4% rebaudioside D by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(b) greater than 4% rebaudioside E by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(c) greater than 15% rebaudioside C by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(d) greater than 15% dulcoside A by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(e) greater than 4% rebaudioside F by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract; or

(f) greater than 3% rebaudioside B by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract.

17. A method for transferring a second sugar moiety to the C-2' of a glucose in a steviol glycoside, said method comprising contacting said steviol glycoside with a 13-monoglucoside beta 1,2-glycosyltransferase or a 19-monoglucoside-beta 1,2-glucosyltransferase and a UDP-sugar under suitable reaction conditions for the transfer of said second sugar moiety to said steviol glycoside, wherein the steviol glycoside optionally can be selected from the group consisting of steviol-13-O-glucoside, rubusoside, stevioside, and rebaudioside A.

18. The method of claim 17, wherein the method is an *in vitro* method, further comprising supplying the UDP-sugar or a cell-free system for regeneration of UDP-sugar.

19. The method of claim 17 or claim 18, wherein said beta 1,2-glycosyltransferase is a UGT91D2 polypeptide, said UGT91D2 polypeptide optionally having at least 90%, 95%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:5, wherein the UGT91D2 polypeptide comprises:

(a) at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5;

(b) an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5;

(c) an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 relative to SEQ ID NO:5;

(d) a tyrosine or phenylalanine at residue 30, a proline or glutamine at residue 93, a serine or valine at residue 99, a tyrosine or phenylalanine at residue 122, a histidine or tyrosine at residue 140, a serine or cysteine at residue 142, an alanine or threonine at residue 148, a methionine at residue 152, an alanine at residue 153, an alanine or serine at residue 156, a glycine at residue 162, a leucine or methionine at residue 195, a glutamic acid at residue 196, a lysine or glutamic acid at residue 199, a leucine or methionine at residue 211, a leucine at residue 213, a serine or phenylalanine at residue 221, a valine or isoleucine at residue 253, a valine or alanine at residue 286, an asparagine or lysine at residue 427, or an alanine at residue 438 and an alanine or threonine at residue 462 relative to SEQ ID NO:5;

(e) the amino acid sequence of SEQ ID NO:95;

(f) the amino acid sequence of SEQ ID NO:5; or

(g) a UGT91D2e or a UGT91D2m polypeptide.

20. The method of any one of claims 17 to 19, wherein the beta 1,2-glycosyltransferase is a UGT79B3 polypeptide having amino acid sequence set forth in SEQ ID NO:150 or a SM12UGT polypeptide such as an IP3GGT polypeptide having amino acid sequence set forth in SEQ ID NO:76 or a UGT94B1 polypeptide having amino acid sequence set forth in SEQ ID NO:78.

21. The method of any one of claims 17 or 18, wherein

(a) said steviol glycoside is rubusoside, said second sugar moiety is glucose, and stevioside is produced upon transfer of said second glucose moiety;

(b) said steviol glycoside is stevioside, said second sugar moiety is glucose, and Rebaudioside E is produced upon transfer of said second glucose moiety;

(c) said steviol glycoside is stevioside, said second sugar moiety is glucose, said stevioside is contacted with said 19-monoglucoside-beta 1,2-glucosyltransferase and a UGT76G1 polypeptide, and Rebaudioside D is produced upon transfer of said second glucose moiety;

(d) said steviol glycoside is steviol-13-O-glucoside, said second sugar moiety is glucose, and steviol-1,2 bioside is produced upon transfer of said second glucose moiety;

(e) said steviol glycoside is steviol-13-O-glucoside, said second sugar moiety is xylose, and steviol-1,2-xylobioside is produced upon transfer of said second sugar moiety;

(f) said steviol glycoside is steviol-13-O-glucoside, said second sugar moiety is rhamnose, and steviol-1,2-rhamnobioside is produced upon transfer of said second sugar moiety; or

(g) said steviol glycoside is rebaudioside A, said second sugar moiety is glucose, and rebaudioside D is produced upon transfer of said second glucose moiety.

22. A method of synthesizing a steviol, steviol glycoside or steviol glycoside composition, wherein a reduced level of Stevia-derived contaminants relative to a plant-derived stevia extract is present, comprising:

(a) growing the recombinant host of any one of claims 1-13 in a culture medium, under conditions in which one or more of said genes are expressed,

wherein growing can include inducing expression of one or more of said genes, such that a compound comprising the steviol or steviol glycoside of interest is synthesized by said host; and

(b) recovering one or more of said compound.

23. The method according to claim 22, comprising:

(a) growing the host of any one of claims 1-13 in a culture medium, under conditions in which said genes are expressed, wherein growing can include inducing expression of one or more of said genes; and

(b) (i) recovering the steviol or the steviol glycoside produced by said host, wherein the steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, or dulcoside A; or

(b) (ii) recovering the steviol glycoside composition produced by said host, wherein said composition is enriched for rebaudioside A, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F or dulcoside A relative to the steviol glycoside composition of a wild-type Stevia plant and has a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract.

24. The method of claim 22 or claim 23, wherein the recombinant host is grown in a fermentor at a defined temperature for a desired period of time.

25. The method of any one of claims 22 to 24, wherein the steviol or steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, and dulcoside A.

26. A recombinant host comprising:

(a) a gene encoding a UGT91D2 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:5; and one or more of:

(b) a gene encoding a UGT85C polypeptide, comprising a UGT85C polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3; and/or

(c) a gene encoding a UGT76G1 polypeptide, comprising a UGT76G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:7; and/or

(d) a gene encoding a UGT74G1 polypeptide, comprising a UGT74G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1;

wherein at least one of said genes is a recombinant gene; and,

wherein the recombinant host produces a steviol or steviol glycoside.

27. The recombinant host of claim 26, wherein the UGT85C polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:3 comprises one or more amino acid substitutions at residues 9, 10, 13, 15, 21, 27, 60, 65, 71, 87, 91,

220, 243, 270, 289, 298, 334, 336, 350, 368, 389, 394, 397, 418, 420, 440, 441, 444, and 471 of SEQ ID NO:3.

28. The recombinant host of claim 26 or claim 27, wherein the UGT76G1 polypeptide having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:7 comprises one or more amino acid substitutions at residues 29, 74, 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, 266, 273, 274, 284, 285, 291, 330, 331, and 346 of SEQ ID NO:7.

29. The recombinant host of any one of claims 26 to 28, wherein said UGT91D2 polypeptide has at least 95% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:5.

30. The recombinant host of any one of claims 26 to 29, wherein said UGT91D2 polypeptide comprises:

(a) at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5;

(b) an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5;

(c) an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 of SEQ ID NO:5;

(d) a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, a threonine at residue 148, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 221, an alanine at residue 286, an asparagine at residue 427, or an alanine at residue 438 of SEQ ID NO:5;

(e) the amino acid sequence of SEQ ID NO:95;

(f) the amino acid sequence of SEQ ID NO:5; or

(g) a UGT91D2e or a UGT91D2m polypeptide.

31. The recombinant host of any one of claims 26 to 30, said host further comprising one or more of the following:

(a) a gene encoding a geranylgeranyl diphosphate synthase;

(b) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;

(c) a gene encoding a kaurene oxidase;

(d) a gene encoding a steviol synthetase;

(e) a gene encoding a truncated HMG-CoA;

(f) a gene encoding a CPR;

(g) a gene encoding a rhamnose synthetase;

(h) a gene encoding a UDP-glucose dehydrogenase; and

(i) a gene encoding a UDP-glucuronic acid decarboxylase.

32. The recombinant host of claim 31, wherein at least one of the genes of (a), (b), (c), (d), (e), (f), (g), (h) or (i) is a recombinant gene.

33. The recombinant host of claim 32, wherein each of the genes of (a), (b), (c) and (d) is a recombinant gene.

34. The recombinant host of any one of claims 26 to 33, wherein the host is a microorganism, comprising a Saccharomycete such as *Saccharomyces cerevisiae*, *Escherichia coli*, or a plant or plant cell, comprising a *Stevia* such as a *Stevia rebaudiana*, *Physcomitrella patens*, or tobacco plant.

35. The recombinant host according to any one of claims 26 to 34, the host further comprising:

(a) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;

(b) a gene encoding a kaurene oxidase;

(c) a gene encoding a steviol synthetase; or

(d) a gene encoding a geranylgeranyl diphosphate synthase,

wherein at least one of said genes is a recombinant gene;

wherein the host produces at least one steviol glycoside such as rebaudioside A, rebaudioside D or rebaudioside E when cultured under conditions in which said genes are expressed,

wherein the host further comprises a gene encoding a rhamnose synthetase and produces at least one steviol glycoside such as rebaudioside C or dulcoside A when cultured under conditions in which said genes are expressed; or

wherein the host further comprises a gene encoding a UDP-glucose dehydrogenase and a gene encoding a UDP-glucuronic acid decarboxylase and produces at least one steviol glycoside such as rebaudioside F when cultured under conditions in which said genes are expressed.

36. The recombinant host according to any one of claims 26 to 35, further comprising exogenous nucleic acids including:

(a) a recombinant gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:121-128;

(b) a recombinant gene encoding an copalyl diphosphate synthase (CDPS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:129-131;

(c) a recombinant gene encoding a kaurene synthase (KS) polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:132-135;

(d) a recombinant gene encoding a kaurene oxidase (KO) polypeptide, comprising KO polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:138-141;

(e) a recombinant gene encoding a steviol synthase polypeptide (KAH), comprising a steviol synthase polypeptide having at least 90% sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs:142-146;

(f) a recombinant gene encoding a cytochrome P450 reductase (CPR) polypeptide, comprising a CPR polypeptide having at least 90% sequence identity to any one of the amino acid sequence set forth in SEQ ID NOs: 147-149;

wherein the recombinant host produces a steviol or a steviol glycoside.

37. The recombinant host of any one of claims 26 to 35, wherein the steviol or the steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, and dulcoside A.

38. The recombinant host of any one of claims 26 to 37 for the production of a steviol or a steviol glycoside, wherein one or more of the genes encoding a polypeptide comprises exogenous nucleic acids.

39. A steviol glycoside composition produced by the recombinant host of any one of claims 26-38, said composition comprising:

(a) greater than 4% rebaudioside D by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(b) greater than 4% rebaudioside E by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(c) greater than 15% rebaudioside C by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(d) greater than 15% dulcoside A by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract;

(e) greater than 4% rebaudioside F by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract; or

(f) greater than 3% rebaudioside B by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract.

40. A method of synthesizing a steviol, steviol glycoside or steviol glycoside composition, wherein a reduced level of Stevia-derived contaminants relative to a plant-derived stevia extract is present, comprising:

(a) growing the recombinant host of any one of claims 26-38 in a culture medium, under conditions in which one or more of said genes are expressed,

wherein growing can include inducing expression of one or more of said genes, such that a compound comprising the steviol or steviol glycoside of interest is synthesized by said host; and

(b) recovering one or more of said compound.

41. The method according to claim 40, comprising:

(a) growing the host of any one of claims 26-38 in a culture medium, under conditions in which said genes are expressed, wherein growing can include inducing expression of one or more of said genes; and

(b) (i) recovering the steviol or the steviol glycoside produced by said host, wherein the steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, or dulcoside A; or

(b) (ii) recovering the steviol glycoside composition produced by said host, wherein said composition is enriched for rebaudioside A, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F or dulcoside A relative to the steviol glycoside composition of a wild-type Stevia plant and has a reduced level of stevia plant-derived contaminants relative to a plant-derived stevia extract.

42. The method of claim 40 or claim 41, wherein the recombinant host is grown in a fermentor at a defined temperature for a desired period of time.

43. The method of any one of claims 40 to 42, wherein the steviol or steviol glycoside comprises steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, and dulcoside A.

WHAT IS CLAIMED IS:

1. A recombinant host comprising:

a) a recombinant gene encoding a UGT91D2 polypeptide such as UGT91D2e or UGT91D2m; and/or

b) a recombinant gene encoding a UGT85C polypeptide having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:3 such as a UGT85C polypeptide comprising one or more amino acid substitutions at residues 9, 10, 13, 15, 21, 27, 60, 65, 71, 87, 91, 220, 243, 270, 289, 298, 334, 336, 350, 368, 389, 394, 397, 418, 420, 440, 441, 444, and 471 of SEQ ID NO:3; and/or

c) a recombinant gene encoding a UGT76G1 polypeptide having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:7, and having one or more amino acid substitutions at residues 29, 74, 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, 266, 273, 274, 284, 285, 291, 330, 331, and 346.

2. The recombinant host of claim 1, wherein said UGT91D2 polypeptide has at least 90%, 95%, or 99% identity to the amino acid sequence set forth in SEQ ID NO:5.

3. The host of claim 1 or 2, wherein said UGT91D2 polypeptide comprises i) at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5; ii) an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5; iii) an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 relative to SEQ ID NO:5; iv) a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, an isoleucine at residue 144, a threonine at residue 148, a leucine at residue 152, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 213, a phenylalanine at residue 221, an alanine at residue 286, a proline at residue 364, a cysteine at residue 384, an asparagine at residue 427, or an alanine at residue 438 relative to SEQ ID NO:5; or v) the amino acid sequence of SEQ ID NO:95; or the amino acid sequence of SEQ ID NO:5.

4. The host of claim 1, wherein said UGT85C polypeptide comprises i) substitutions at residues 13, 60, and 270 of SEQ ID NO:3; ii) substitutions at residues 60 and 87 of SEQ ID NO:3; iii) substitutions at residues 65, 71, 220, 243, and 270 of SEQ ID NO:3; iv) substitutions at residues 65, 71, 220, 243, 270, and 441 of SEQ ID NO:3; v) substitutions at residues 65, 71, 220, 389, and 394 of SEQ ID NO:3; vi) substitutions at residues 65, 71, 270, and 289 of SEQ ID NO:3; vii) substitutions at residues 15 and 65 of SEQ ID NO:3; viii) substitutions at residues 65 and 270 of SEQ ID NO:3; ix) substitutions at residues 65 and 440 of SEQ ID NO:3; x) substitutions at residues 65 and 441 of SEQ ID NO:3; xi) substitutions at residues 65 and 418 of SEQ ID NO:3; xii) substitutions at residues 220, 243, 270, and 334 of SEQ ID NO:3; xiii) substitutions at residues 270 and 289 of SEQ ID NO:3; or xiv) substitutions at residues 13, 15, 60, 270, 289, and 418 of SEQ ID NO:3.

5. The host of claim 1, wherein said UGT76 polypeptide has i) substitutions at amino acid residues 74 , 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, and 291; ii) substitutions at residues 74 , 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, 266, 273, 274, 284, 285, and 291; or iii) substitutions at residues 74 , 87, 91, 116, 123, 125, 126, 130, 145, 192, 193, 194, 196, 198, 199, 200, 203, 204, 205, 206, 207, 208, 266, 273, 274, 284, 285, 291, 330, 331, and 346.

6. The host of any one of claims 1-5, said host further comprising one or more of
(i) a gene encoding a UGT74G1 polypeptide such as a recombinant gene encoding a UGT74G1 polypeptide;
(ii) a gene encoding a geranylgeranyl diphosphate synthase;
(iii) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;
(iv) a gene encoding a kaurene oxidase;
(v) a gene encoding a steviol synthetase;
(vi) a gene encoding a truncated HMG-CoA;
(vii) a gene encoding a CPR;

(viii) a gene encoding a rhamnose synthetase;
(ix) a gene encoding a UDP-glucose dehydrogenase; and
(x) a gene encoding a UDP-glucuronic acid decarboxylase.

7. The host of claim 6, wherein at least one of the genes of (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), or (x) is a recombinant gene, for example each of the genes of (i), (ii), (iii), and (iv) is a recombinant gene.

8. An isolated nucleic acid:

a) encoding a polypeptide having at least 90%, 95%, or 99% identity to the amino acid sequence set forth in SEQ ID NO:5, such as i) a polypeptide comprising at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5; ii) a polypeptide comprising an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5; iii) a polypeptide comprising an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 of SEQ ID NO:5; or iv) a polypeptide comprising a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, an isoleucine at residue 144, a threonine at residue 148, a leucine at residue 152, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 213, a phenylalanine at residue 221, an alanine at residue 286, a proline at residue 364, a cysteine at residue 384, an asparagine at residue 427, or an alanine at residue 438 relative to SEQ ID NO:5; or

b) encoding a polypeptide having greater than 90 % sequence identity to the amino acid sequence of UGT91D2e or UGT91D2m, excluding the amino acid sequence of SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14; or

c) having greater than 90 % sequence identity to one of the nucleotide sequences set forth in SEQ ID NOs: 18-25, 34-36, 4-43, 48, 49, 52-55, 60-64, 70-72, 77, or 79.

9. An isolated polypeptide having an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO:5 or an isolated polypeptide having greater than 90 % sequence identity to the amino acid sequence of UGT91D2e or UGT91D2m, excluding the amino acid sequence of SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

10. A recombinant host, said host comprising:
(i) a gene encoding a geranylgeranyl diphosphate synthase such as a geranylgeranyl diphosphate synthase having greater than 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 121-128;
(ii) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase such as a copalyl diphosphate synthase having greater than 90 % sequence identity to the amino acid sequence set forth in SEQ ID NOs: 129-131 and a gene encoding a kaurene synthase such as a kaurene synthase having greater than 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 132-135;
(iii) a gene encoding a kaurene oxidase such as a kaurene oxidase having greater than 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 138-141; and
(iv) a gene encoding a steviol synthetase such as a steviol synthetase having greater than 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 142-146;
wherein at least one of said genes is a recombinant gene.

11. The host of claim 10, wherein said host produces steviol when cultured under conditions in which each of said genes is expressed and optionally accumulates steviol to at least 1 mg/L of culture medium when cultured under said conditions.

12. The host of any one of claims 10 or 11, further comprising one or more of:
a gene encoding a truncated HMG-CoA;
a gene encoding a CPR;
a gene encoding a UGT74G1 polypeptide;
a gene encoding UGT85C2 polypeptide;
a gene encoding a UGT76G1 polypeptide;
a gene encoding a UGT91D2 or SM12UGT polypeptide;

a gene encoding a deoxyxylulose 5-phosphate synthase (DXS);
a gene encoding a D-1-deoxyxylulose 5-phosphate reductoisomerase (DXR);
a gene encoding a 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (CMS);
a gene encoding a 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK);
a gene encoding a 4-diphosphocytidyl-2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MCS);
a gene encoding a 1-hydroxy-2-methyl-2(E)-butenyl 4-diphosphate synthase (HDS);
a gene encoding a 1-hydroxy-2-methyl-2(E)-butenyl 4-diphosphate reductase (HDR);
a gene encoding a acetoacetyl-CoA thiolase;
a gene encoding a truncated HMG-CoA reductase;
a gene encoding a mevalonate kinase;
a gene encoding a phosphomevalonate kinase; and
a gene encoding a mevalonate pyrophosphate decarboxylase; wherein expression of one or more of said genes can be inducible.

13. The host of claim 12, wherein said UGT91D2 polypeptide can be UGT91D2e or UGT91D2m, or wherein said SM12UGT polypeptide is an IP3GGT polypeptide or a UGT94B1 polypeptide, or wherein said UGT91D2 polypeptide comprises i) at least one amino acid substitution at residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5; ii) an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5; iii) an arginine at residue 206, a cysteine at residue 207, and an arginine at residue 343 relative to SEQ ID NO:5; iv) a phenylalanine at residue 30, a glutamine at residue 93, a valine at residue 99, a phenylalanine at residue 122, a tyrosine at residue 140, a cysteine at residue 142, an isoleucine at residue 144, a threonine at residue 148, a leucine at residue 152, an alanine at residue 153, a serine at residue 156, a methionine at residue 195, a glutamic acid at residue 196, a glutamic acid at residue 199, a methionine at residue 211, a phenylalanine at residue 213, a phenylalanine at residue 221, an alanine at residue 286, a proline at residue 364, a cysteine at residue 384, an asparagine at residue 427, or an alanine at residue 438 relative to SEQ ID NO:5; or v) the amino acid sequence of SEQ ID NO:95; or the amino acid sequence of SEQ ID NO:5.

14. The host of any one of claims 10-13, wherein said host produces at least one steviol glycoside when cultured under conditions in which each of said genes is expressed, such as a steviol glycoside selected from the group consisting of steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, and dulcoside A, and wherein said steviol glycoside can accumulate to at least 1 mg/liter, 10 mg/liter, or 20 mg/liter of culture medium when cultured under said conditions.

15. The host of any one of claims 1-5 or 10-14, wherein said host is a microorganism, a Saccharomycete such as *Saccharomyces cerevisiae*, *Escherichia coli*, or a plant or a plant cell such as a *Stevia*, *Stevia rebaudiana*, *Physcomitrella*, or tobacco plant or plant cell.

16. A method of producing steviol, a steviol glycoside, or a steviol glycoside composition, comprising:

a) growing the host of anyone of claims 6, 7, or 10-15 in a culture medium, under conditions in which said genes are expressed, wherein growing can include inducing expression of one or more of said genes; and

b i) recovering the steviol or steviol glycoside produced by said host, wherein the steviol glycoside can be, for example, steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, or dulcoside A; or

b ii) recovering the steviol glycoside composition produced by said host, wherein said composition is enriched for rebaudioside A, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F or dulcoside A relative to the steviol glycoside composition of a wild-type Stevia plant and has a reduced level of stevia plant-derived contaminants relative to a stevia extract.

17. A recombinant *Saccharomyces* strain comprising:
(i) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or
(ii) a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;
wherein said strain produces ent-kaurene upon expression of said copalyl diphosphate synthase and said kaurene synthase.

18. A recombinant host, said host comprising:

(i) a recombinant gene encoding a UGT91D2;

(ii) a recombinant gene encoding a UGT74G1;

iii) a recombinant gene encoding a UGT85C2;

(iv) a recombinant gene encoding a UGT76G1; and

(v) optionally

(a) a gene encoding a bifunctional copalyl diphosphate synthase and kaurene synthase, or a gene encoding a copalyl diphosphate synthase and a gene encoding a kaurene synthase;

(b) a gene encoding a kaurene oxidase;

(c) a gene encoding a steviol synthetase; and

(d) a gene encoding a geranylgeranyl diphosphate synthase,

wherein said host produces at least one steviol glycoside such as rebaudioside A, rebaudioside D or rebaudioside E when cultured under conditions in which each of said genes is expressed, wherein said host further can include a gene encoding a rhamnose synthetase, and produce at least one steviol glycoside such as rebaudioside C or dulcoside A when cultured under conditions in which each of said genes is expressed; or

wherein said host further can include a gene encoding a UDP-glucose dehydrogenase; and a gene encoding a UDP-glucuronic acid decarboxylase, and produce at least one steviol glycoside such as rebaudioside F when cultured under conditions in which each of said genes is expressed.

19. A steviol glycoside composition produced by the host of claim 18, said composition having a) greater than 4% rebaudioside D by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a stevia extract; b) greater than 4% rebaudioside E by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a stevia extract; c) greater than 15% rebaudioside C by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a stevia extract; d) greater than 15% dulcoside A by weight total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a stevia extract; or e) greater than 4% rebaudioside F by weight

total steviol glycosides and a reduced level of stevia plant-derived contaminants relative to a stevia extract.

20. A method for transferring a second sugar moiety to the C-2' of a glucose in a steviol glycoside, said method comprising contacting said steviol glycoside with a 13-monoglucoside beta 1,2 glycosyltransferase or a 19-monoglucoside-beta 1,2-glucosyltransferase and a UDP-sugar under suitable reaction conditions for the transfer of said second sugar moiety to said steviol glycoside, wherein the steviol glycoside optionally can be selected from the group consisting of steviol-13-O-glucoside, rubusoside, stevioside, and rebaudioside A.

21. The method of claim 20, wherein said beta 1,2 glycosyltransferase is a UGT91D2 polypeptide, said UGT91D2 polypeptide optionally having at least 90%, 95%, or 99% identity to the amino acid sequence set forth in SEQ ID NO:5, such as

a UGT91D2 polypeptide comprising an amino acid substitution at one or more residues selected from the group consisting of residues 1-19, 27-38, 44-87, 96-120, 125-141, 159-184, 199-202, 215-380, or 387-473 of SEQ ID NO:5 or

a UGT91D2 polypeptide comprising an amino acid substitution at one or more residues selected from the group consisting of residues 30, 93, 99, 122, 140, 142, 144, 148, 152, 153, 156, 195, 196, 199, 206, 207, 211, 213, 221, 286, 343, 364, 384, 427, and 438 of SEQ ID NO:5.

22. The method of claim 20, wherein said beta 1,2 diglycosyltransferase is a SM12UGT polypeptide such as an IP3GGT polypeptide or a UGT94B1 polypeptide.

23. The method of any one of claims 20-22, wherein

i) said steviol glycoside is rubusoside, said second sugar moiety is glucose, and stevioside is produced upon transfer of said second glucose moiety;

ii) said steviol glycoside is stevioside, said second sugar moiety is glucose, and Rebaudioside E is produced upon transfer of said second glucose moiety;

iii) said steviol glycoside is stevioside, said second sugar moiety is glucose, said stevioside is contacted with said 19-monoglucoside-beta 1,2-glucosyltransferase and a UGT76G1 polypeptide, and Rebaudioside D is produced upon transfer of said second glucose moiety;

iv) said steviol glycoside is steviol-13-*O*-glucoside, said second sugar moiety is glucose, and steviol-1,2 bioside is produced upon transfer of said second glucose moiety;

v) said steviol glycoside is steviol-13-*O*-glucoside, said second sugar moiety is xylose, and steviol-1,2-xylobioside is produced upon transfer of said second sugar moiety;

vi) said steviol glycoside is steviol-13-*O*-glucoside, said second sugar moiety is rhamnose, and steviol-1,2-rhamnobioside is produced upon transfer of said second sugar moiety; or

vii) said steviol glycoside is rebaudioside A, said second sugar moiety is glucose, and rebaudioside D is produced upon transfer of said second glucose moiety.