WHAT IS CLAIMED IS:

1. A recombinant host comprising a recombinant gene encoding a polypeptide having at least approximately 80% identity to the amino acid sequence set forth in SEQ ID NO:152;

said host optionally comprising one or more of the following:

(a) a recombinant gene encoding a UGT91d2e or UGT91d2m polypeptide, for example, the UGT91d2e polypeptide having a substitution at residues 211 and 286 of SEQ ID NO:5, such as UGT91D2e-b, which contains a methionine at residue 211 and an alanine at residue 286 of SEQ ID NO:5;

(b) a recombinant gene encoding a steviol synthase polypeptide having approximately 80% identity to the amino acid sequence set forth in FIG. 12C (SEQ ID NO:164) (the KAHe1);

(c) a recombinant gene encoding an *ent*-copalyl diphosphate synthase (EC 5.5.1.13; CDPS) polypeptide lacking a chloroplast transit peptide;

(d) one or more exogenous nucleic acids encoding a sucrose transporter such as a *SUC1* sequence from *Arabidopsis thaliana* and a sucrose synthase such as a *SUS1* sequence from *Coffea arabica*, *Arabidopsis thaliana*, or *S. rebaudiana*, particularly wherein expression of the one or more exogenous nucleic acids and expression of a glucosyltransferase results in increased levels of uridine diphosphate glucose (UDP-glucose) in the host;

(e) a recombinant gene encoding a CPR polypeptide having at least approximately 80% sequence identity to the *S. rebaudiana* CPR amino acid sequence set forth in FIG. 13 (SEQ ID NO:170);

(f) a nucleic acid sequence, said nucleic acid comprising a heterologous insert sequence operably linked to an open reading frame, wherein the heterologous insert sequence has the general formula (I):

$$-X_1-X_2-X_3-X_4-X_5- \quad (I)$$

wherein X_2 comprises at least 4 consecutive nucleotides complementary to at least 4 consecutive nucleotides of X_4, wherein X_3 comprises zero nucleotides or one or more nucleotides forming a hairpin loop, wherein X_1 and X_5 each individually consists of zero nucleotides or one or more nucleotides, wherein the open reading frame encodes a squalene synthase (EC 2.5.1.21).

(g) one or more exogenous nucleic acids encoding a *S. rebaudiana* KO polypeptide, the polypeptide comprising a sequence with at least approximately 80% identity to SEQ ID NO:138, and an *A. thaliana* CPR polypeptide such as SEQ ID NO:168;

(h) a recombinant gene encoding an *A. thaliana* KS polypeptide, the polypeptide comprising a sequence with at least approximately 80% identity to SEQ ID NO:156;

(i) a codon optimized recombinant gene encoding a UGT85C; and

(j) a recombinant gene encoding a GGPPS.

2. A recombinant host comprising a recombinant gene encoding a polypeptide having at least approximately 80% identity to the amino acid sequence set forth in FIG. 12C (SEQ ID NO:164), for example, about 85% identity, about 90% identity, about 95% identity, or about 100% identity.

3. A recombinant host cell comprising a nucleic acid sequence, said nucleic acid comprising a heterologous insert sequence operably linked to an open reading frame, wherein the heterologous insert sequence has the general formula (I):

$$-X_1-X_2-X_3-X_4-X_5-$$

wherein X_2 comprises at least 4 consecutive nucleotides complementary to at least 4 consecutive nucleotides of X_4,

wherein X_3 comprises zero nucleotides or one or more nucleotides forming a hairpin loop,

wherein X_1 and X_5 each individually consists of zero nucleotides, or one or more nucleotides,

wherein the open reading frame encodes a squalene synthase (EC 2.5.1.21).

4. The cell according to claim 3, wherein the nucleic acid sequence comprises

i) a promoter sequence operably linked to

ii) a heterologous insert sequence operably linked to

iii) an open reading frame operably linked to

iv) a transcription termination signal,

wherein the heterologous insert sequence has the general formula (I):

$-X_1-X_2-X_3-X_4-X_5-$

wherein X_2 comprises at least 4 consecutive nucleotides complementary to and forming a stem secondary structure element with at least 4 consecutive nucleotides of X_4, and wherein X_3 comprises nucleotides forming a hairpin loop between X_2 and X_4, and wherein X_1 and X_5 individually and optionally comprises one or more nucleic acids, and

wherein the open reading frame upon expression encodes a polypeptide sequence having at least approximately 70% identity to a squalene synthase (EC 2.5.1.21) or a biologically active fragment thereof, said fragment having at least approximately 70% sequence identity to said squalene synthase in a range of overlap of at least 100 amino acids.

5. The cell according to any one of claim 3 to 4, wherein the cell furthermore comprises a heterologous nucleic acid encoding GGPPS operably linked to a nucleic acid sequence directing expression of GGPPS in said cell.

6. A recombinant host comprising a recombinant gene encoding a recombinant gene encoding an *ent*-copalyl diphosphate synthase (EC 5.5.1.13; CDPS) polypeptide lacking a chloroplast transit peptide.

7. The host of claim 6, wherein the polypeptide is a *Zea mays* CDPS lacking 50 amino acids from the carboxy terminus of the unmodified *Zea mays* CDPS polypeptide;

8. The host of any one of claims 1-7, said host further comprising one or more of:

a recombinant gene encoding a UGT85C polypeptide having approximately 90% or greater identity to the amino acid sequence set forth in SEQ ID NO:3;

a recombinant gene encoding a UGT76G polypeptide having approximately 90% or greater identity to the amino acid sequence set forth in SEQ ID NO:7;

a gene encoding a UGT74G1 polypeptide such as a recombinant gene; and

a gene encoding a UGT91D2 functional homolog.

9. The host of any one of claims 1-8, said host further comprising one or more of:

(i) a gene encoding a geranylgeranyl diphosphate synthase;

(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 at least approximately 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 at least approximately 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 132-135 or SEQ ID NO:156.

(iii) a gene encoding a kaurene oxidase such as a kaurene oxidase having at least approximately 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 138-141;

(iv) a gene encoding a steviol synthetase such as a steviol synthetase having at least approximately 90 % sequence identity to one of the amino acid sequences set forth in SEQ ID NOs: 142-146 and 164;

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

(vi) a gene encoding a CPR;

(vii) a gene encoding a rhamnose synthetase;

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

(ix) a gene encoding a UDP-glucuronic acid decarboxylase, particularly wherein at least one of the genes of (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), or (ix) is a recombinant gene.

10. The host of any one of claims 1-9, wherein said host is a microorganism, for example, a Saccharomycete such as *Saccharomyces cerevisiae*.

11. A method of producing a steviol glycoside, comprising:

a) growing the host of any one of claims 1-10 in a culture medium, under conditions in which said genes are expressed, for example, inducing expression of one or more of said genes; and

b) recovering said steviol glycoside produced by said host.

12. The method of claim 11, wherein the steviol glycoside is produced in a permeabilized host cell which has been transformed with a EUGT11 polypeptide.

13. The method of claim 12, wherein said steviol glycoside is Rebaudioside A, and wherein Rebaudioside D is produced upon transfer of a glucose moiety from a sugar donor to Rebaudioside A.

14. A method for increasing the level of UDP-glucose and reducing the level of UDP in a cell, the method comprising expressing in the cell a recombinant sucrose synthase and a recombinant sucrose transporter, in a medium comprising sucrose, wherein the cell is deficient in sucrose degradation, or wherein said expressing results in a decreased level of UDP and an increased level of UDP-glucose in the cell, such that glycosylation in the cell is increased.

Claims

1. A recombinant host, wherein the host is a microorganism, a plant or a plant cell, capable of producing one or more steviol glycosides comprising a recombinant gene encoding a polypeptide having at least 80% sequence identity to the amino acid sequence set forth in SEQ ID NO: 152; the host further comprising one or more of:
   (a) a recombinant gene encoding a UGT85C2 polypeptide having approximately 90% or greater identity to the amino acid sequence set forth in SEQ ID NO:3;
   (b) a recombinant gene encoding a UGT76G1 polypeptide having approximately 90% or greater identity to the amino acid sequence set forth in SEQ ID NO:7;
   (c) a gene encoding a UGT74G1 polypeptide such as a recombinant gene;
   (d) a recombinant gene encoding a UGT91D2 functional homolog;
   (e) a recombinant gene encoding a steviol synthase polypeptide (KAH);
   (f) a recombinant gene encoding an ent-copalyl diphosphate synthase (CDPS) polypeptide;
   (g) one or more recombinant genes encoding a sucrose transporter (SUC1) and a sucrose synthase (SUS1);
   (h) a recombinant gene encoding a cytochrome P450 reductase (CPR) polypeptide;
   (i) a nucleic acid sequence, said nucleic acid comprising a heterologous insert sequence operably linked to an open reading frame encoding a squalene synthase (SQS), wherein the heterologous insert sequence has a formula (I):

       -X1-X2-X3-X4-X5- (I)

       wherein X2 comprises at least 4 consecutive nucleotides complementary to at least 4 consecutive nucleotides of X4, wherein X3 comprises zero nucleotides or one or more nucleotides forming a hairpin loop, wherein X1 and X5 each individually consists of zero nucleotides or one or more nucleotides;
   (j) one or more recombinant genes encoding a kaurene oxidase (KO) polypeptide;
   (k) a recombinant gene encoding a kaurene synthase (KS) polypeptide; or
   (l) a recombinant gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide.

2. The recombinant host of claim 1, wherein the UGT91D2 functional homolog comprises a UGT91d2e polypeptide having a substitution at residues 211 and 286 of SEQ ID NO:5 or

a UGT91D2e-b polypeptide having a methionine at residue 211 and an alanine at residue 286 of SEQ ID NO:5.

3. The recombinant host of claim 1 or 2, wherein the KAH polypeptide has at least 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:164.

4. The recombinant host of any one of claims 1 to 3, wherein the CDPS polypeptide is lacking a chloroplast transit peptide.

5. The recombinant host of any one of claims 1 to 4, wherein the expression of the one or more recombinant genes encoding the sucrose transporter and expression of the sucrose synthase results in increased levels of uridine diphosphate glucose (UDP-glucose) in the host.

6. The recombinant host of any one of claims 1 to 5, wherein the CPR polypeptide has at least 80% sequence identity to the S. rebaudiana CPR amino acid sequence set forth in SEQ ID NO:170.

7. The recombinant host of any one of claims 1 to 6, wherein
   (a) the GGPPS polypeptide has at least 90% identity to the amino acid sequence set forth in SEQ ID NO:127;
   (b) the KO polypeptide has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:138;
   (c) the CPR polypeptide has at least 90% sequence identity to the amino acid sequences set forth in SEQ ID NO:168 and SEQ ID NO:170; or
   (d) the KS polypeptide has at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:156.

8. The recombinant host of any one of claims 1 to 7, further comprising one or more of: a gene encoding an 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.

9. The recombinant host of any one of claims 1 to 8, said recombinant host further comprising one or more of:

(i) a gene encoding a bifunctional CDPS and KS or a gene encoding a CDPS and a gene encoding a KS;

(ii) a gene encoding a rhamnose synthetase;

(iii) a gene encoding a UDP-glucose dehydrogenase; or

(iv) a gene encoding a UDP-glucuronic acid decarboxylase,

wherein at least one of the genes of (i), (ii), (iii), or (iv), is a recombinant gene.

10. The recombinant host of claim 9, wherein the CDPS has at least 90% sequence identity to one of the amino acid sequences set forth in SEQ ID NOs:129-131.

11. The recombinant host of claims 9 or 10, wherein the KS has at least 90% sequence identity to one of the amino acid sequences set forth in SEQ ID NOs:132-135 or SEQ ID NO:156.

12. The recombinant host of any one of claims 9 to 11, wherein the KO has at least 90% sequence identity to one of the amino acid sequences set forth in SEQ ID NOs:138-141.

13. The recombinant host of any one of claims 9 to 12, wherein the KAH has at least 90% sequence identity to one of the amino acid sequences set forth in SEQ ID NOs:142-146 or 164.

14. The recombinant host of any one of claims 1 to 13, 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.

15. A method of producing a steviol glycoside, comprising:

(a) growing the recombinant host of any one of claims 1 to 14 in a culture medium, under conditions in which the genes discussed in any one of claims 1 to 14 are expressed; and

(b) recovering from said culture medium said steviol glycoside produced by said recombinant host.

16. The method of claim 15, wherein the steviol glycoside comprises steviol-13-O-glucoside, steviol-1,2-bioside, steviol-1,3-bioside, steviol-19-O-glucoside, stevioside, 1,3 stevioside, rubusoside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, or dulcoside A.

17. The method of claim 15, wherein said steviol glycoside is Rebaudioside D, and wherein Rebaudioside D is produced upon transfer of a glucose moiety from a sugar donor to Rebaudioside A.