Claims

1. A method for producing a steviol glycoside product from steviol glycoside intermediates, comprising:

providing a bacterial cell expressing one or more UDP-dependent glycosyl transferase enzymes (UGT enzymes) intracellularly, wherein the UGT enzymes glycosylate steviol and steviol glycoside substrates, and wherein the bacterial cell comprises the following genetic modifications:

deletion, inactivation, or reduced activity or expression of UDP-sugar hydrolase and one or more UDP-galactose biosynthesis enzymes;

deletion, inactivation, or reduced activity or expression of glucose-6-phosphate isomerase; and

overexpression of phosphoglucomutase and UTP-glucose-1-phosphate uridylyltransferase;

incubating the bacterial cell with a *stevia* leaf extract or fraction thereof comprising the steviol glycoside intermediates thereby glycosylating the steviol glycoside intermediates to the steviol glycoside product by enzymatic transfer of one or more glucosyl groups from UDP-glucose cofactor, and

recovering the steviol glycoside product.

2. The method of claim 1, wherein the steviol glycoside intermediates comprise one or more of stevioside, steviolbioside, rebaudioside A, dulcoside A, dulcoside B, rebaudioside C, and rebaudioside F.

3. The method of claim 2, wherein the extract comprises stevioside, steviolbioside, and Rebaudioside A as prominent components.

4. The method of claim 3, wherein the steviol glycoside product comprises RebM.

5. The method of claim 2, wherein the steviol glycoside product comprises RebK, RebC+1, and/or RebC+2.

6. The method of claim 1, wherein the bacterial cell is *Escherichia* spp., *Bacillus* spp., *Rhodobacter* spp., *Zymomonas* spp., or *Pseudomonas* spp.

7. The method of claim 6, wherein the bacterial cell is *Escherichia coli, Bacillus subtilis, Rhodobacter capsulatus, Rhodobacter sphaeroides, Zymomonas mobilis*, or *Pseudomonas putida.*

8. The method of claim 7, wherein the bacterial cell is *E. coli.*

9. The method of claim 8, wherein the bacterial cell comprises the genetic modifications: ushA and galETKM are deleted; pgi is deleted; and pgm and galU are overexpressed.

10. The method of claim 1, wherein the bacterial cell expresses a 1-3′ glycosylating UGT enzyme.

11. The method of claim 10, wherein the 1-3′ glycosylating UGT enzyme comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17.

12. The method of claim 1, wherein the bacterial cell expresses a 1-2′ glycosylating UGT enzyme.

13. The method of claim 1, wherein the bacterial cell expresses a UGT enzyme that glycosylates the C13 hydroxyl of steviol or steviol glycoside substrate.

14. The method of claim 1, wherein the bacterial cell expresses a UGT enzyme that glycosylates the C19 hydroxyl of steviol or steviol glycoside substrate.

15. The method of claim 1, wherein the bacterial cell expresses a 1-3′ glycosylating UGT enzyme and a 1-2′ glycosylating UGT enzyme.

16. The method of claim 15, wherein the bacterial cell expresses a 1-3′ glycosylating UGT enzyme, a 1-2′ glycosylating UGT enzyme, a C19 O-glycosylating UGT enzyme, and a C13 O-glycosylating UGT enzyme.

17. The method of claim 16, wherein the bacterial cell expresses: a SrUGT85C2 (SEQ ID NO: 1) or derivative thereof having at least 90% sequence identity thereto; MbUGT1,2 (SEQ ID NO: 9) or derivative thereof having at least 90% sequence identity thereto; SrUGT74G1 (SEQ ID NO: 2) or derivative thereof having at least 90% sequence identity thereto; and an enzyme selected from SrUGT76G1 (SEQ ID NO: 3) or derivative thereof having at least 90% sequence identity thereto, MbUGT1-3_1 (SEQ ID NO: 15) or derivative thereof having at least 90% sequence identity thereto, MbUGT1-3_1.5 (SEQ ID NO: 16) or derivative thereof having at least 90% sequence identity thereto, and MbUGT1-3_2 (SEQ ID NO: 17) or derivative thereof having at least 90% sequence identity thereto.

18. The method of claim 1, wherein the UGT enzymes are integrated into the chromosome of the bacterial cell or are expressed extrachromosomally.

19. The method of claim 1, wherein galETKM genes are inactivated, deleted, or reduced in expression.

20. The method of claim 1, wherein the bacterial cell has a deletion, inactivation, or reduced activity or expression of trehalose-6-phosphate synthase.

21. The method of claim 1, wherein the bacterial cell has a deletion, inactivation, or reduced activity or expression of UDP-glucose 6-dehydrogenase.

22. The method of claim 1, wherein the bacterial cell has an overexpression or increased activity or expression of ycjU (βphosphoglucomutase) and *Bifidobacterium* bifidum ugpA.

23. The method of claim 1, wherein the bacterial cell has one or more genetic modifications that increase flux to the pentose phosphate pathway (PPP), and which is an overexpression of *E. coli* zwf.

24. The method of claim 1, wherein the bacterial cell has one or more genetic modifications that increase UTP production and recycling, and which are selected from increased expression or activity of *E. coli* pyrH (UMP kinase), *E. coli* cmk (cytidylate kinase), *E. coli* adk (adenylate kinase), and *E. coli* ndk (nucleoside diphosphate kinase).

25. The method of claim 1, wherein the bacterial cell has one or more genetic modifications that increase UDP production, and which are selected from overexpression or increased activity of: upp (uracil phosphoribosyltransferase), pyrH (UMP kinase), cmk (cytidylate kinase); dctA (C4 dicarboxylate/orotate:H+symporter), pyrE (orotate phosphoribosyltransferase), pyrH (UMP kinase) and cmk (cytidylate kinase).

26. The method of claim 1, wherein the bacterial cell has one or more genetic modifications to remove or reduce regulation of glucose uptake, which include deletion, inactivation, or reduced expression of sgrS small regulatory RNA.

27. The method of claim 1, wherein the bacterial cell has one or more genetic modifications that reduce conversion of glucose-1-phosphate to TDP-glucose, which include a deletion, inactivation, or reduced expression or activity of one or more dTDP-glucose pyrophosphorylase genes.

28. The method of claim 1, wherein the bacterial cell has one or more genetic modifications that reduce conversion of glucose-1-phosphate to ADP-glucose, and which include deletion, inactivation, or reduced expression or activity of glucose-1-phosphate adenylyltransferase.

29. The method of claim 1, wherein the method results in at least 40% conversion of steviol glycoside intermediate to steviol glycoside product by weight.

30. The method of claim 29, wherein the method results in a least 50% conversion of steviol glycoside intermediate to steviol glycoside product by weight.

31. The method of claim 30, wherein the method results in at least 75% conversion of steviol glycoside intermediate to steviol glycoside product by weight.

32. The method of claim 1, wherein a ratio of RebM to RebD of at least 5:1 is recovered as the steviol glycoside product.

33. The method of claim 1, wherein the method is performed by batch fermentation, continuous fermentation, or semi-continuous fermentation.

34. The method of claim 33, wherein the method is performed by fed batch fermentation.

35. The method of claim 34, wherein the steviol glycoside intermediates are incubated with the bacterial cell for about 72 hours or less.

36. The method of claim 2, wherein the steviol glycosideglycosylated product comprises RebD, RebE, RebI, or RebB.