4. SUMMARY

[0003] Provided herein are genetically modified host cells, compositions, and methods for the improved production of Reb M. These compositions and methods are based in part on the expression of certain heterologous ABC-transporters in host cells that have been genetically modified to produce steviol glycosides such as Reb M. These ABC-transporters are capable of transporting certain steviol glycosides, preferably Reb M and/or the related high molecular weight steviol glycoside rebaudioside D (Reb D), out of the cytosol either into the extracellular space or into the lumen of subcellular organelles, for example the yeast vacuole. The sequestration of certain steviol glycosides like Reb D and Reb M increases the efficiency of the steviol glycoside metabolic pathway by relieving the product inhibition caused by the accumulation of steviol glycosides.

[0003a] In one aspect, the present disclosure refers to a genetically modified host cell capable of producing one or more steviol glycosides comprising a heterologous nucleic acid encoding an ATP-binding cassette (ABC)-transporter, wherein the genetically modified host cell with the ABC-transporter produces more total steviol glycoside (TSG) compared to thea genetically modified host cell lackingin the absence of the ABC-transporter, wherein the ABC-transportergenetically modified host cell comprisesing: an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, and wherein the ABC-transporter is capable of transporting steviol glycoside.

[0004] In one example of the disclosure, provided herein are genetically modified host cells and methods of their use for the production of industrially useful compounds. In one example, provided herein is a genetically modified host cell capable of producing one or more steviol glycosides where the host cell contains a heterologous nucleic acid encoding an ABC-transporter having an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

[0005] In one example of the disclosure the ABC-transporter has an amino acid sequence having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30. In another example the genetically modified host cells of the disclosure contain nucleic acids encoding geranylgeranyl pyrophosphate synthase (GGPPS), ent-copalyl pyrophosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene 19-oxidase (KO), ent-kaurenoic acid 13-hydroxylase (KAH), cytochrome p450 reductase (CPR), and one or more UDP-glucosyltransferases (UGT). In a further example the one or more UDP-glucosyltransferases (UGT) are selected from EUGT11, UGT85C2, UGT74G1, UGT91D_like3, UGT76G1, and UGT40087. In a further example of the disclosure the geranylgeranyl pyrophosphate synthase (GGPPS) has an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 9, the ent-copalyl pyrophosphate synthase (CPS) has an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 10, the ent-kaurene synthase (KS) has an amino acid sequence having at least 80% sequence identity to

molecule, rubusoside, to provide stevioside. A UGT91D is also referred to as UGT91D2, UGT91D2e, or UGT91D-like3. Illustrative examples of UGT91D enzymes include those of *Stevia rebaudiana* (*e.g.*, those of UGT sequence with accession no. ACE87855.1, US 2014/0329281 A1, WO 2016/038095 A2, and SEQ ID NO:17). Nucleic acids encoding these enzymes are useful in the cells and methods provided herein. In certain embodiments, provided herein are cells and methods using a nucleic acid having at least 80%, 85%, 90%, or 95% sequence identity to at least one of these UGT91D nucleic acids. In certain embodiments, provided herein are cells and methods using a nucleic acid that encodes a polypeptide having at least 80%, 85%, 90%, or 95% sequence identity to at least one of these UGT91D enzymes.

6.4.12 Uridine Diphosphate-Dependent Glycosyl Transferase capable of converting Reb A to Reb D (UGT_{AD})

[00107] A uridine diphosphate-dependent glycosyl transferase (UGT_{AD}) is capable of transferring a glucose moiety to the C-2' position of the 19-O-glucose of Reb A to produce Reb D. A UGT_{AD} is also capable of transferring a glucose moiety to the C-2' position of the 19-O-glucose of stevioside to produce Reb E . Useful examples of UGTs include Os_UGT_91C1 from *Oryza sativa* (also referred to as EUGT11 in Houghton-Larsen *et al.*, WO 2013/022989 A2; XP_015629141.1) and Sl_UGT_101249881 from *Solanum lycopersicum* (also referred to as UGTSL2 in Markosyan *et al.*, WO2014/193888 A1; XP_004250485.1). Further useful UGTs include UGT40087 (XP_004982059.1; as described in WO 2018/031955), sr.UGT_9252778, Bd_UGT10840 (XP_003560669.1), Hv_UGT_V1 (BAJ94055.1), Bd_UGT10850 (XP_010230871.1), and Ob_UGT91B1_like (XP_006650455.1). Any UGT or UGT variant can be used in the compositions and methods described herein. Nucleic acids encoding these enzymes are useful in the cells and methods provided herein. In certain embodiments, provided herein are cells and methods using a nucleic acid having at least 80%, 85%, 90%, or 95% sequence identity to at least one of the UGTs. In certain embodiments, provided herein are cells and methods using a nucleic acid that encodes a polypeptide having at least 80%, 85%, 90%, or 95% sequence identity to at least one of these UGTs. In certain embodiments, provided herein are a nucleic acid that encodes a UGT variant described herein.

WHAT IS CLAIMED:

1. A genetically modified host cell capable of producing one or more steviol glycosides comprising a heterologous nucleic acid encoding an ATP-binding cassette (ABC)-~~ABC~~-transporter, wherein the genetically modified host cell with the ABC-transporter produces more total steviol glycoside (TSG) compared to ~~a~~ the genetically modified host cell ~~lacking~~ in the absence of the ABC-transporter, wherein the ABC-transporter ~~the genetically modified host cell~~ comprises~~ing:~~ an amino acid sequence ~~having at least 80% sequence identity to an amino acid sequence~~ selected from the group consisting of SEQ ID NO: 7, ~~SEQ ID NO: 1,~~ SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, and wherein the ABC-transporter is capable of transporting steviol glycoside.

~~2. The genetically modified host cell of claim 1, wherein the ABC-transporter comprises an amino acid sequence having a sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 8.~~

~~3.~~2. The genetically modified host cell of any of the preceding claims, further comprising a nucleic acid encoding geranylgeranyl pyrophosphate synthase (GGPPS), ent-copalyl pyrophosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene 19-oxidase (KO), ent-kaurenoic acid 13-hydroxylase (KAH), cytochrome p450 reductase (CPR), and one or more UDP-glucosyltransferases (UGT).

~~4.~~3. The genetically modified host cell of claim ~~3~~2, wherein the one or more UDP-glucosyltransferases (UGT) are selected from the group consisting of UDP glycosyltransferase 85C2~~UGT85C2~~, UDP glycosyltransferase 74G1~~UGT74G1~~, UDP-glycosyltransferase 91D~~UGT91D_like3~~, UDP glycosyltransferase 76G1~~UGT76G1~~, Os glycosyltransferase 91C1~~EUGT11~~, and UDP- glycosyltransferase UGT40087.

~~5.~~4. The genetically modified host cell of claim ~~4~~3, wherein the geranylgeranyl pyrophosphate synthase (GGPPS) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO: 9, the ent-copalyl pyrophosphate synthase (CPS) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO:

10, the ent-kaurene synthase (KS) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO: 11, the ent-kaurene 19-oxidase (KO) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO: 12, the ent-kaurenoic acid 13-hydroxylase (KAH) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO: 13, the cytochrome p450 reductase (CPR) comprises an amino acid sequence ~~having at least 80% sequence identity to~~of SEQ ID NO: 14, and the one or more UDP-glucosyltransferases (UGT) comprise an amino acid sequence ~~having at least 80% sequence identity to an amino acid sequence~~ selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO:18, SEQ ID NO: 19.

~~6.~~5. The genetically modified host cell of claim ~~5~~4, wherein the geranylgeranyl pyrophosphate synthase (GGPPS) comprises an amino acid sequence of SEQ ID NO: 9, the ent-copalyl pyrophosphate synthase (CPS) comprises an amino acid sequence of SEQ ID NO: 10, the ent-kaurene synthase (KS) comprises an amino acid sequence of SEQ ID NO: 11, the ent-kaurene 19-oxidase (KO) comprises an amino acid sequence of SEQ ID NO: 12, the ent-kaurenoic acid 13-hydroxylase (KAH) comprises an amino acid sequence of SEQ ID NO: 13, the cytochrome p450 reductase (CPR) comprises an amino acid sequence of SEQ ID NO: 14, and the one or more UDP-glucosyltransferases (UGT) comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO:18, SEQ ID NO: 19.

~~7.~~6. The genetically modified host cell of any of the preceding claims, wherein the host cell is selected from a bacterial cell, a fungal cell, an algal cell, an insect cell, and a plant cell.

~~8.~~7. The genetically modified host cell of claim ~~7~~6, wherein the host cell is a *Saccharomyces cerevisiae* cell.

~~9.~~8. The genetically modified host cell of any of the preceding claims, wherein the ABC-transporter comprises an amino acid sequence having the sequence of SEQ ID NO: 7.

~~10.~~9. The genetically modified host cell of claim ~~9~~8, wherein the ABC-transporter comprises one or more amino acid substitutions relative to the amino acid sequence of SEQ ID NO: 7~~.~~

~~11.~~ ~~The genetically modified host cell of claim 10~~, wherein the one or more amino acid substitutions are selected from V666A, Y942N, L956P, and E1320V.

~~12.~~10. The genetically modified host cell of any of the preceding claims, wherein the one or more steviol glycosides is selected from the group consisting of Rebaudioside~~Reb~~ A, Rebaudioside~~Reb~~ B, Rebaudioside~~Reb~~ D, Rebaudioside~~Reb~~ E, and Rebaudioside~~Reb~~ M.

~~13.~~11. The genetically modified host cell of claim ~~12~~10, wherein the one or more steviol glycosides comprises Rebaudioside~~Reb~~ M.

~~14. A polynucleotide comprising a nucleotide sequence of the heterologous nucleic acid of any of the preceding claims.~~

~~15.~~12. The genetically modified host cell of any of the preceding claims~~polynucleotide of claim 14~~, wherein the nucleotide sequence of the heterologous nucleic comprises a coding sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, wherein the coding sequence is operably linked to a heterologous promoter.

~~16.~~13. A method for producing steviol or one or more steviol glycosides comprising the steps:

(a) culturing a population of the host cells of any one of claims 1 to ~~13~~ 11 in a medium with a carbon source under conditions suitable for making steviol or one or more steviol glycosides to yield a culture broth; and

(b) recovering said steviol or one or more steviol glycosides from the culture broth.

~~17.~~14. A method for producing Rebaudioside~~Reb~~ M comprising the steps:

(a) culturing a population of the host cells of any one of claims 1 to ~~13~~ 11 in a medium with a carbon source under conditions suitable for making Rebaudioside~~Reb~~ M to yield a culture broth; and

(b) recovering said Rebaudioside~~Reb~~ M compound from the culture broth.

~~18. The genetically modified host cell of claim 1 or 2, wherein at least 50% of the one or more steviol glycosides accumulate within a lumen of an organelle.~~

~~19. The genetically modified host cell of claim 1 or 2, wherein at least 50% of the one or more steviol glycosides accumulate extracellularly.~~