

(86) Date de dépôt PCT/PCT Filing Date: 2015/08/07
(87) Date publication PCT/PCT Publication Date: 2016/02/18
(85) Entrée phase nationale/National Entry: 2017/02/06
(86) N° demande PCT/PCT Application No.: EP 2015/068314
(87) N° publication PCT/PCT Publication No.: 2016/023844
(30) Priorité/Priority: 2014/08/11 (US62/035,902)

(51) Cl.Int./Int.Cl. *C12N 1/19* (2006.01),
C07H 15/256 (2006.01), *C12N 1/21* (2006.01),
C12N 15/29 (2006.01), *C12N 15/31* (2006.01),
C12N 15/52 (2006.01), *C12N 15/63* (2006.01),
C12N 15/81 (2006.01), *C12P 19/56* (2006.01)

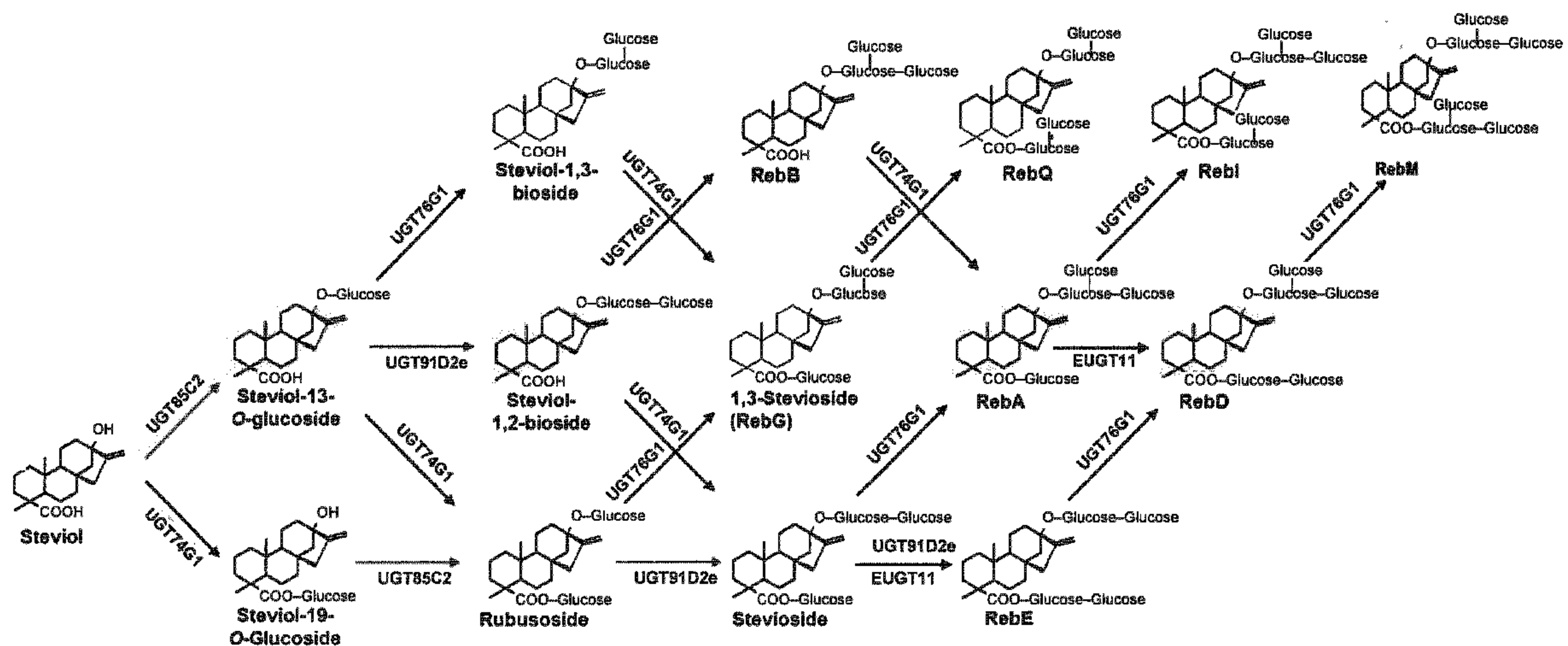
(71) Demandeur/Applicant:
EVOLVA SA, CH

(72) Inventeurs/Inventors:
DOUCHIN, VERONIQUE, DK;
MIKKELSEN, MICHAEL DALGAARD, DK;
MOLLER-HANSEN, IBEN, DK

(74) **Agent:** BENOIT & COTE INC.

(54) Titre : PRODUCTION DE GLYCOSIDES DE STEVIOL DANS DES HOTES DE RECOMBINAISON
(54) Title: PRODUCTION OF STEVIOL GLYCOSIDES IN RECOMBINANT HOSTS

Figure 1



(57) **Abrégé/Abstract:**

The invention relates to recombinant microorganisms and methods for producing steviol glycosides and steviol glycoside precursors.



(10) International Publication Number
WO 2016/023844 A1

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

[illegible]

(57) Abstract: The invention relates to recombinant microorganisms and methods for producing steviol glycosides and steviol glycoside precursors.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 78

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 78

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

PRODUCTION OF STEVIOL GLYCOSIDES IN RECOMBINANT HOSTS

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This disclosure relates generally to the recombinant production of steviol glycosides such as rebaudioside A (RebA), rebaudioside B (RebB), rebaudioside D (RebD), and rebaudioside M (RebM) by recombinant hosts such as recombinant microorganisms and isolation methods thereof. In particular, this disclosure relates to modifications to transport systems in a recombinant host to increase production of such steviol glycosides and/or transport of such steviol glycosides into the culture medium.

Description of Related Art

[0002] Sweeteners are well known as ingredients used most commonly in the food, beverage, or confectionary industries. The sweetener can either be incorporated into a final food product during production or for stand-alone use, when appropriately diluted, as a tabletop sweetener or an at-home replacement for sugars in baking. Sweeteners include natural sweeteners such as sucrose, high fructose corn syrup, molasses, maple syrup, and honey and artificial sweeteners such as aspartame, saccharine, and sucralose. Stevia extract is a natural sweetener that can be isolated and extracted from a perennial shrub, *Stevia rebaudiana*. Stevia is commonly grown in South America and Asia for commercial production of stevia extract. Stevia extract, purified to various degrees, is used commercially as a high intensity sweetener in foods and in blends or alone as a tabletop sweetener.

[0003] Chemical structures for several steviol glycosides are shown in Figure 1, including the diterpene steviol and various steviol glycosides. Extracts of the Stevia plant generally comprise rebaudiosides and other steviol glycosides that contribute to the sweet flavor, although the amount of each steviol glycoside often varies, *inter alia*, among different production batches.

[0004] As recovery and purification of steviol glycosides from the Stevia plant have proven to be labor intensive and inefficient, there remains a need for a recombinant production system that can produce high yields of desired steviol glycosides, such as RebD and RebM.

SUMMARY OF THE INVENTION

[0005] It is against the above background that the present invention provides certain advantages and advancements over the prior art.

[0006] In particular, the invention provides a recombinant host capable of synthesizing a steviol glycoside, comprising a gene encoding a transporter polypeptide and/or a gene encoding a transcription factor polypeptide that regulates expression of at least one transporter gene; wherein expression of the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide that regulates expression of at least one transporter gene is modified and the recombinant host transports at least a portion of the synthesized steviol glycoside from the host into a culture medium.

[0007] In some aspects of the recombinant host disclosed herein, the gene encoding the transporter polypeptide is an endogenous gene.

[0008] In some aspects of the recombinant host disclosed herein, the transporter polypeptide comprises an ATP-binding cassette (ABC) transporter, a major facilitator superfamily (MFS) transporter, an amino acid/auxin permease (AAP) family transporter, ATPase transporter, a sulfate permease (SulP) family transporter, a lysosomal cystine transporter (LCT) family transporter, a Ca^{2+} :cation antiporter (CaCA) family transporter, an amino acid-polyamine-organocation (APC) superfamily transporter, a multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) transporter, a ZRT/IRT-like protein (ZIP) metal transporter family transporter, a mitochondrial protein translocase (MPT) family transporter, a voltage-gated ion channel (VIC) family transporter, a monovalent cation:proton antiporter-2 (CPA2) family transporter, a ThrE family of putative transmembrane amino acid efflux transporter, an oligopeptide transporter (OPT) family transporter, a K^{+} transporter (Trk) family transporter, a bile acid:Na symporter (BASS) family transporter, a drug/metabolite transporter (DMT) superfamily transporter, a mitochondrial carrier (MC) family transporter, an auxin efflux carrier (AEC) family transporter, an ammonia channel transporter (Amt) family transporter, a metal ion (Mn^{2+} -iron) transporter (Nramp) family transporter, a transient receptor potential Ca^{2+} channel (TRP-CC) family transporter, an arsenical resistance-3 (ACR3) family transporter, a nucleobase:cation symporter-1 (NCS1) family transporter, an inorganic phosphate transporter (PiT) family transporter, an arsenite-antimonite (ArsAB) efflux family transporter, an IISF family of transporter, a glycerol uptake (GUP) family transporter, a metal ion transport (MIT) family transporter, a copper transport (Ctr) family or a cation diffusion facilitator (CDF) family transporter.

[0009] In some aspects of the recombinant host disclosed herein, the modified expression comprises modified expression comprises:

- (a) overexpressing the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide; or
- (b) deleting the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide.

[0010] In some aspects of the recombinant host disclosed herein, the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide has an activity that is increased.

[0011] In some aspects of the recombinant host disclosed herein, one or more of the genes encoding the transporter polypeptide and/or one or more of the genes encoding the transcription factor polypeptide are overexpressed.

[0012] In some aspects of the recombinant host disclosed herein, the transporter polypeptide and/or transcription polypeptide comprise YAL067C set forth in SEQ ID NO:14, YBL089W set forth in SEQ ID NO:15, YBL099W set forth in SEQ ID NO:16, YBR008C set forth in SEQ ID NO:86, YBR021W set forth in SEQ ID NO:87, YBR043C set forth in SEQ ID NO:88, YBR180W set forth in SEQ ID NO:13, YBR241C set forth in SEQ ID NO:17, YBR287W set forth in SEQ ID NO:89, YBR294W set forth in SEQ ID NO:18, YBR295W set forth in SEQ ID NO:90, YBR296C set forth in SEQ ID NO:91, YCL038C set forth in SEQ ID NO:92, YCL069W set forth in SEQ ID NO:19, YCR011C set forth in SEQ ID NO:93, YCR028C set forth in SEQ ID NO:20, YCR075C set forth in SEQ ID NO:21, YDL054C set forth in SEQ ID NO:94, YDL100C set forth in SEQ ID NO:95, YDL128W set forth in SEQ ID NO:22, YDL185W set forth in SEQ ID NO:23, YDL194W set forth in SEQ ID NO:24, YDL210W set forth in SEQ ID NO:25, YDL245C set forth in SEQ ID NO:96, YDL247W set forth in SEQ ID NO:97, YDR011W set forth in SEQ ID NO:98, YDR061W set forth in SEQ ID NO:26, YDR093W set forth in SEQ ID NO:27, YDR292C set forth in SEQ ID NO:99, YDR338C set forth in SEQ ID NO:28, YDR406W set forth in SEQ ID NO:29, YDR497C set forth in SEQ ID NO:100, YDR536W set forth in SEQ ID NO:30, YEL006W set forth in SEQ ID NO:101, YEL027W set forth in SEQ ID NO:102, YEL031W set forth in SEQ ID NO:31, YEL065W set forth in SEQ ID NO:103, YER019C-A set forth in SEQ ID NO:104, YER053C set forth in SEQ ID NO:105, YER119C set forth in SEQ ID NO:106, YER166W set forth in SEQ ID NO:32, YFL011W set forth in SEQ ID NO:33, YFL028C set forth in SEQ ID NO:107, YFR045W set forth in SEQ ID NO:108, YGL006W set forth in SEQ ID NO:34, YGL013C set forth in SEQ ID NO:35, YGL084C set forth in SEQ ID NO:109, YGL104C set forth in SEQ ID NO:110, YGL114W set forth in SEQ ID NO:111, YGL167C set forth in SEQ ID NO:112, YGL255W set forth in SEQ ID NO:36, YGR125W set forth in SEQ ID NO:37, YGR181W set forth in SEQ ID NO:38, YGR217W set forth in SEQ ID NO:39, YGR224W set forth in SEQ ID

NO:40, YGR257C set forth in SEQ ID NO:113, YGR281W set forth in SEQ ID NO:41, YHL016C set forth in SEQ ID NO:42, YHL035C set forth in SEQ ID NO:114, YHL036W set forth in SEQ ID NO:115, YHR002W set forth in SEQ ID NO:116, YHR096C set forth in SEQ ID NO:117, YIL006W set forth in SEQ ID NO:118, YIL088C set forth in SEQ ID NO:43, YIL120W set forth in SEQ ID NO:119, YIL121W set forth in SEQ ID NO:120, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJL094C set forth in SEQ ID NO:45, YJL108C set forth in SEQ ID NO:46, YJL133W set forth in SEQ ID NO:122, YJL212C set forth in SEQ ID NO:47, YJL219W set forth in SEQ ID NO:123, YJR106W set forth in SEQ ID NO:48, YJR160C set forth in SEQ ID NO:49, YKL016C set forth in SEQ ID NO:124, YKL050C set forth in SEQ ID NO:125, YKL064W set forth in SEQ ID NO:50, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKL209C set forth in SEQ ID NO:128, YKR039W set forth in SEQ ID NO:129, YKR050W set forth in SEQ ID NO:51, YKR105C set forth in SEQ ID NO:52, YKR106W set forth in SEQ ID NO:53, YLR411W set forth in SEQ ID NO:130, YLR447C set forth in SEQ ID NO:54, YML038C set forth in SEQ ID NO:131, YML116W set forth in SEQ ID NO:55, YMR034C set forth in SEQ ID NO:56, YMR056C set forth in SEQ ID NO:57, YMR166C set forth in SEQ ID NO:132, YMR253C set forth in SEQ ID NO:58, YMR279C set forth in SEQ ID NO:133, YNL003C set forth in SEQ ID NO:134, YNL065W set forth in SEQ ID NO:59, YNL070W set forth in SEQ ID NO:60, YNL083W set forth in SEQ ID NO:61, YNL095C set forth in SEQ ID NO:62, YNL121C set forth in SEQ ID NO:63, YNL142W set forth in SEQ ID NO:64, YNL268W set forth in SEQ ID NO:135, YNR055C set forth in SEQ ID NO:136, YOL020W set forth in SEQ ID NO:65, YOL075C set forth in SEQ ID NO:66, YOL077W-A set forth in SEQ ID NO:67, YOL122C set forth in SEQ ID NO:68, YOL158C set forth in SEQ ID NO:137, YOR079C set forth in SEQ ID NO:69, YOR087W set forth in SEQ ID NO:70, YOR092W set forth in SEQ ID NO:71, YOR100C set forth in SEQ ID NO:138, YOR130C set forth in SEQ ID NO:72, YOR153W set forth in SEQ ID NO:139, YOR222W set forth in SEQ ID NO:73, YOR271C set forth in SEQ ID NO:140, YOR273C set forth in SEQ ID NO:141, YOR291W set forth in SEQ ID NO:74, YOR306C set forth in SEQ ID NO:75, YOR307C set forth in SEQ ID NO:142, YOR316C set forth in SEQ ID NO:76, YOR332W set forth in SEQ ID NO:143, YOR334W set forth in SEQ ID NO:77, YOR348C set forth in SEQ ID NO:144, YPL036W set forth in SEQ ID NO:145, YPL078C set forth in SEQ ID NO:78, YPL270W set forth in SEQ ID NO:79, YPL274W set forth in SEQ ID NO:80, YPR003C set forth in SEQ ID NO:81, YPR011C set forth in SEQ ID NO:82, YPR058W set forth in SEQ ID NO:83, YPR128C set forth in SEQ ID NO:84, or YPR201W set forth in SEQ ID NO:85.

[0013] In some aspects of the recombinant host disclosed herein, YBR043C set forth in SEQ ID NO:88, YDL100C set forth in SEQ ID NO:95, YDL054C set forth in SEQ ID NO:94,

YDL128W set forth in SEQ ID NO:22, YDL198C set forth in SEQ ID NO:146, YDR061W set forth in SEQ ID NO:26, YDR536W set forth in SEQ ID NO:30, YEL027W set forth in SEQ ID NO:102, YFL054C set forth in SEQ ID NO:147, YGL167C set forth in SEQ ID NO:112, YGR181W set forth in SEQ ID NO:38, YHL016C set forth in SEQ ID NO:42, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJR106W set forth in SEQ ID NO:48, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKR039W set forth in SEQ ID NO:129, YMR034C set forth in SEQ ID NO:56, YMR166C set forth in SEQ ID NO:132, YOL122C set forth in SEQ ID NO:68, YOR079C set forth in SEQ ID NO:69, YPL270W set forth in SEQ ID NO:79, and/or YPR011C set forth in SEQ ID NO:82 are overexpressed.

[0014] In some aspects, the recombinant host further comprises:

- (a) one or more genes encoding a sucrose transporter and a sucrose synthase;
- (b) a gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide;
- (c) a gene encoding an ent-copalyl diphosphate synthase (CDPS) polypeptide;
- (d) a gene encoding a kaurene synthase (KS) polypeptide;
- (e) a gene encoding a kaurene oxidase (KO) polypeptide;
- (f) a gene encoding a steviol synthase (KAH) polypeptide;
- (g) a gene encoding a cytochrome P450 reductase (CPR) polypeptide;
- (h) a gene encoding a UGT85C2 polypeptide;
- (i) a gene encoding a UGT76G1 polypeptide;
- (k) a gene encoding a UGT91D2 functional homolog; and/or
- (l) a gene encoding a EUGT11 polypeptide;

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

wherein the host is capable of producing one or more of RebA, RebB, RebD and/or RebM.

[0015] In some aspects of the recombinant host disclosed herein, at least one of the genes is codon optimized for expression in the host.

[0016] In some aspects of the recombinant host disclosed herein, at least one of the genes is codon optimized for expression in *Saccharomyces cerevisiae*.

[0017] In some aspects of the recombinant host disclosed herein,

- (a) the GGPPS polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:149;
- (b) the CDPS polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:150;
- (c) the KO polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:152;
- (d) the KS polypeptide comprises a polypeptide having at least 40% identity to an amino acid sequence set forth in SEQ ID NO:151;
- (e) the KAH polypeptide comprises a polypeptide having at least 60% identity to an amino acid sequence set forth in SEQ ID NO:154;
- (f) the CPR polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:153 and/or a polypeptide having at least 65% identity to an amino acid sequence set forth in SEQ ID NO:155;
- (g) the UGT85C2 polypeptide comprises a polypeptide having at least 55% identity to an amino acid sequence set forth in SEQ ID NO:156;
- (h) the UGT76G1 polypeptide comprises a polypeptide having at least 50% identity to an amino acid sequence set forth in SEQ ID NO:158;
- (i) the UGT74G1 polypeptide comprises a polypeptide having at least 55% identity to an amino acid sequence set forth in SEQ ID NO:157;
- (j) the a UGT91D2 functional homolog comprises a UGT91D2e-b polypeptide having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:159; and
- (k) the EUGT11 polypeptide comprises a polypeptide having at least 65% identity to an amino acid sequence set forth in SEQ ID NO:148.

[0018] In some aspects, the recombinant host disclosed herein comprises a microorganism that is a plant cell, a mammalian cell, an insect cell, a fungal cell, or a bacterial cell.

[0019] In some aspects, the bacterial cell comprises *Escherichia* bacteria cells, *Lactobacillus* bacteria cells, *Lactococcus* bacteria cells, *Cornebacterium* bacteria cells, *Acetobacter* bacteria cells, *Acinetobacter* bacteria cells, or *Pseudomonas* bacterial cells.

[0020] In some aspects, the fungal cell is a yeast cell.

[0021] In some aspects, the yeast cell is a cell from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Candida glabrata*, *Ashbya gossypii*, *Cyberlindnera jadinii*, *Pichia pastoris*, *Kluyveromyces lactis*, *Hansenula polymorpha*, *Candida boidinii*, *Arxula adeninivorans*, *Xanthophyllomyces dendrorhous*, or *Candida albicans* species.

[0022] In some aspects, the yeast cell is a *Saccharomycete*.

[0023] In some aspects, the yeast cell is a cell from the *Saccharomyces cerevisiae* species.

[0024] The invention further provides a method of producing a steviol glycoside, comprising:

- (a) growing the recombinant host disclosed herein in a culture medium, under conditions in which the genes comprising recombinant host disclosed herein are expressed,

wherein the steviol glycoside is synthesized by the host; and

- (b) optionally isolating the steviol glycoside.

[0025] In some aspects of the methods disclosed herein, the steviol glycoside is RebA, RebB, RebD, and/or RebM, and wherein:

- (a) RebA is capable of being synthesized in the recombinant host disclosed herein expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2;
- (b) RebB is capable of being synthesized in the recombinant host disclosed herein expressing UGT85C2, UGT76G1, and UGT91D2;
- (c) RebD is capable of being synthesized in the recombinant host disclosed herein expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11; and
- (d) RebM is capable of being synthesized in the recombinant host disclosed herein expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11.

[0026] In some aspects of the methods disclosed herein a gene encoding YBR043C set forth in SEQ ID NO:88, YDL100C set forth in SEQ ID NO:95, YDL054C set forth in SEQ ID NO:94, YDL128W set forth in SEQ ID NO:22, YDL198C set forth in SEQ ID NO:146, YDR061W set forth in SEQ ID NO:26, YDR536W set forth in SEQ ID NO:30, YEL027W set forth in SEQ ID NO:102, YFL054C set forth in SEQ ID NO:147, YGL167C set forth in SEQ ID NO:112, YGR181W set forth in SEQ ID NO:38, YHL016C set forth in SEQ ID NO:42, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJR106W set forth in SEQ ID NO:48, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKR039W set forth in SEQ ID NO:129, YMR034C set forth in SEQ ID NO:56, YMR166C set forth in SEQ ID NO:132, YOL122C set forth in SEQ ID NO:68, YOR079C set forth in SEQ ID NO:69, YPL270W set forth in SEQ ID NO:79, and/or YPR011C set forth in SEQ ID NO:82 is overexpressed.

[0027] In some aspects of the methods disclosed herein the steviol glycoside is produced at a concentration of between about 500 mg/L to about 10,000 mg/L.

[0028] The invention further provides a method of increasing production or transport of a steviol glycoside into a culture medium, comprising:

- (a) growing the recombinant host disclosed herein in a culture medium, under conditions in which the genes comprising the host disclosed herein are expressed,

wherein the steviol glycoside is synthesized by the host; and

- (b) optionally isolating the steviol glycoside.

[0029] In some aspects of the methods disclosed herein, the steviol glycoside is RebA, RebB, RebD, and/or RebM.

[0030] The invention further provides a method increasing production of steviol or a steviol glycoside in a recombinant host, comprising modifying expression of a gene encoding a transporter polypeptide and/or a gene encoding a transcription that regulates expression of at least one transporter gene, wherein the host is capable of transporting at least a portion of the produced steviol or a steviol glycoside from the host into a culture medium.

[0031] These and other features and advantages of the present invention will be more fully understood from the following detailed description of the invention taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

DESCRIPTION OF DRAWINGS

[0032] Figure 1 shows the chemical structures and synthesis pathways for various steviol glycosides.

[0033] Figure 2 is a bar graph of the amount (μM) of RebA, RebB, RebD, or RebM in the supernatant of a steviol glycoside-producing strain overexpressing transporter genes YGR181W (SEQ ID NO:38) or YDR061W (SEQ ID NO:26), compared to a control steviol glycoside-producing strain. See Example 4.

[0034] Figure 3A and Figure 3B are bar graphs of the amount (mg/L) of RebA, RebD, or RebM in the supernatant (Figure 3A) or total culture (Figure 3B) of a YGR181W (SEQ ID NO:38) or YDR061W (SEQ ID NO:26) overexpressing strain, compared to a control steviol glycoside-producing strain. See Example 4.

[0035] Figure 4A shows levels of 13-SMG (total levels and supernatant levels; $\mu\text{M}/\text{OD}_{600}$), Figure 4B shows levels of RebA (total levels and supernatant levels; $\mu\text{M}/\text{OD}_{600}$), Figure 4C shows levels of RebB (total levels and supernatant levels; $\mu\text{M}/\text{OD}_{600}$), Figure 4D shows levels of RebD (total levels and supernatant levels; $\mu\text{M}/\text{OD}_{600}$), and Figure 4E shows levels of RebM (total levels and supernatant levels; $\mu\text{M}/\text{OD}_{600}$) in a steviol glycoside-producing *S. cerevisiae* strain with a genomically integrated transporter gene. The genomically integrated transporter genes of Figures 4A-E are YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), YJL093C (SEQ ID NO:44), YJR106W (SEQ ID NO:48), YMR166C (SEQ ID NO:132), YIL166C (SEQ ID NO:121), YKL120W (SEQ ID NO:126), YDL054C (SEQ ID NO:94), YDL128W (SEQ ID NO:22), YDR536W (SEQ ID NO:30), YGL167C (SEQ ID NO:112), YKL146W (SEQ ID NO:127), YKR039W (SEQ ID NO:129), YOL122C (SEQ ID NO:68), and YPR011C (SEQ ID NO:82). See Example 6.

[0036] Figure 5A shows supernatant levels of RebA, RebB, RebD, and RebM (in $\mu\text{M}/\text{OD}_{600}$) of a steviol glycoside-producing strain overexpressing YMR166C (SEQ ID NO:132), YEL027W (SEQ ID NO:102), YKL120W (SEQ ID NO:126), YIL166C (SEQ ID NO:121), YJR106W (SEQ ID NO:48), YJL093C (SEQ ID NO:44), and YBR043C (SEQ ID NO:88) by the USER cloning system. Figure 5B shows total levels of RebA, RebB, RebD, and RebM (in $\mu\text{M}/\text{OD}_{600}$) of a steviol glycoside-producing strain overexpressing YMR166C (SEQ ID NO:132), YEL027W (SEQ ID NO:102), YKL120W (SEQ ID NO:126), YIL166C (SEQ ID NO:121), YJR106W (SEQ ID NO:48), YJL093C (SEQ ID NO:44), and YBR043C (SEQ ID NO:88) by the USER cloning system.

DETAILED DESCRIPTION

[0037] All publications, patents and patent applications cited herein are hereby expressly incorporated by reference in their entirety for all purposes.

[0038] Before describing the present invention in detail, a number of terms will be defined. As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. For example, reference to “a nucleic acid” means one or more nucleic acids.

[0039] It is noted that terms like “preferably,” “commonly,” and “typically” are not utilized herein to limit the scope of the claimed invention or to imply that certain features are critical, essential, or even important to the structure or function of the claimed invention. Rather, these terms are merely intended to highlight alternative or additional features that can or cannot be utilized in a particular embodiment of the present invention.

[0040] For the purposes of describing and defining the present invention it is noted that the term “substantially” is utilized herein to represent the inherent degree of uncertainty that can be attributed to any quantitative comparison, value, measurement, or other representation. The term “substantially” is also utilized herein to represent the degree by which a quantitative representation can vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

[0001] Methods well known to those skilled in the art can be used to construct genetic expression constructs and recombinant cells according to this invention. These methods include in vitro recombinant DNA techniques, synthetic techniques, in vivo recombination techniques, and polymerase chain reaction (PCR) techniques. See, for example, techniques as described in Green & Sambrook, 2012, MOLECULAR CLONING: A LABORATORY MANUAL, Fourth Edition, Cold Spring Harbor Laboratory, New York; Ausubel *et al.*, 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, New York, and PCR Protocols: A Guide to Methods and Applications (Innis *et al.*, 1990, Academic Press, San Diego, CA).

[0041] As used herein, the terms “polynucleotide,” “nucleotide,” “oligonucleotide,” and “nucleic acid” can be used interchangeably to refer to nucleic acid comprising DNA, RNA, derivatives thereof, or combinations thereof.

[0042] As used herein, the terms “microorganism,” “microorganism host,” “microorganism host cell,” “host cell,” “recombinant host,” “recombinant microorganism host,” and “recombinant host cell” can be used interchangeably. As used herein, the term “recombinant host” is intended to refer to a host, the genome of which has been augmented

by at least one DNA sequence. Such DNA sequences include but are not limited to genes that are not naturally present, DNA sequences that are not normally transcribed into RNA or translated into a protein ("expressed"), and other genes or DNA sequences which one desires to introduce into the non-recombinant host. It will be appreciated that typically the genome of a recombinant host described herein is augmented through stable introduction of one or more recombinant genes. Generally, introduced DNA is not originally resident in the host that is the recipient of the DNA, but it is within the scope of this disclosure to isolate a DNA segment from a given host, and to subsequently introduce one or more additional copies of that DNA into the same host, e.g., to enhance production of the product of a gene or alter the expression pattern of a gene. In some instances, the introduced DNA will modify or even replace an endogenous gene or DNA sequence by, e.g., homologous recombination or site-directed mutagenesis. Suitable recombinant hosts include microorganisms.

[0043] As used herein, the term "recombinant gene" refers to a gene or DNA sequence that is introduced into a recipient host, regardless of whether the same or a similar gene or DNA sequence may already be present in such a host. "Introduced," or "augmented" in this context, is known in the art to mean introduced or augmented by the hand of man. Thus, a recombinant gene can be a DNA sequence from another species or can be a DNA sequence that originated from or is present in the same species but has been incorporated into a host by recombinant methods to form a recombinant host. It will be appreciated that a recombinant gene that is introduced into a host can be identical to a DNA sequence that is normally present in the host being transformed and is introduced to provide one or more additional copies of the DNA to thereby permit overexpression or modified expression of the gene product of that DNA. Said recombinant genes are particularly encoded by cDNA.

[0044] As used herein, the term "engineered biosynthetic pathway" refers to a biosynthetic pathway that occurs in a recombinant host, as described herein, and does not naturally occur in the host.

[0045] As used herein, the term "endogenous" gene refers to a gene that originates from and is produced or synthesized within a particular organism, tissue, or cell. In some embodiments, the endogenous gene is a yeast transporter. In some embodiments, the transporter is endogenous to *S. cerevisiae*, including, but not limited to *S. cerevisiae* strain S288C. In some embodiments, an endogenous yeast transporter gene is overexpressed. As used herein, the term "overexpress" is used to refer to the expression of a gene in an organism at levels higher than the level of gene expression in a wild type organism. See, e.g., Prelich, 2012, Genetics 190:841-54. In some embodiments, an endogenous yeast transporter gene is deleted. See, e.g., Giaever & Nislow, 2014, Genetics 197(2):451-65. As used herein, the terms "deletion," "deleted," "knockout," and "knocked out" can be used

interchangeably to refer to an endogenous gene that has been manipulated to no longer be expressed in an organism, including, but not limited to, *S. cerevisiae*. In some embodiments, a deleted/knocked out gene is a transporter gene or a transcription factor gene that regulates expression of a transporter gene.

[0046] As used herein, the terms “heterologous sequence” and “heterologous coding sequence” are used to describe a sequence derived from a species other than the recombinant host. In some embodiments, the recombinant host is an *S. cerevisiae* cell, and a heterologous sequence is derived from an organism other than *S. cerevisiae*. A heterologous coding sequence, for example, can be from a prokaryotic microorganism, a eukaryotic microorganism, a plant, an animal, an insect, or a fungus different than the recombinant host expressing the heterologous sequence. In some embodiments, a coding sequence is a sequence that is native to the host.

[0047] A “selectable marker” can be one of any number of genes that complement host cell auxotrophy, provide antibiotic resistance, or result in a color change. Linearized DNA fragments of the gene replacement vector then are introduced into the cells using methods well known in the art (see below). Integration of the linear fragments into the genome and the disruption of the gene can be determined based on the selection marker and can be verified by, for example, PCR or Southern blot analysis. Subsequent to its use in selection, a selectable marker can be removed from the genome of the host cell by, e.g., Cre-LoxP systems (see, e.g., Gossen *et al.*, 2002, *Ann. Rev. Genetics* 36:153-173 and U.S. 2006/0014264). Alternatively, a gene replacement vector can be constructed in such a way as to include a portion of the gene to be disrupted, where the portion is devoid of any endogenous gene promoter sequence and encodes none, or an inactive fragment of, the coding sequence of the gene.

[0048] As used herein, the terms “variant” and “mutant” are used to describe a protein sequence that has been modified at one or more amino acids, compared to the wild type sequence of a particular protein.

[0049] As used herein, the term “inactive fragment” is a fragment of the gene that encodes a protein having, e.g., less than about 10% (e.g., less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, or 0%) of the activity of the protein produced from the full-length coding sequence of the gene. Such a portion of a gene is inserted in a vector in such a way that no known promoter sequence is operably linked to the gene sequence, but that a stop codon and a transcription termination sequence are operably linked to the portion of the gene sequence. This vector can be subsequently

linearized in the portion of the gene sequence and transformed into a cell. By way of single homologous recombination, this linearized vector is then integrated in the endogenous counterpart of the gene with inactivation thereof.

[0050] As used herein, the term “steviol glycoside” refers to Rebaudioside A (RebA) (CAS # 58543-16-1), Rebaudioside B (RebB) (CAS # 58543-17-2), Rebaudioside C (RebC) (CAS # 63550-99-2), Rebaudioside D (RebD) (CAS # 63279-13-0), Rebaudioside E (RebE) (CAS # 63279-14-1), Rebaudioside F (RebF) (CAS # 438045-89-7), Rebaudioside M (RebM) (CAS # 1220616-44-3), Rubusoside (CAS # 63849-39-4), Dulcoside A (CAS # 64432-06-0), Rebaudioside I (RebI) (MassBank Record: FU000332), Rebaudioside Q (RebQ), 1,2-Stevioside (CAS # 57817-89-7), 1,3-Stevioside (RebG), 1,2-Bioside (MassBank Record: FU000299), 1,3-Bioside, Steviol-13-O-glucoside (13-SMG), Steviol-19-O-glucoside (19-SMG), a tri-glucosylated steviol glycoside, a tetra-glucosylated steviol glycoside, a penta-glucosylated steviol glycoside, a hexa-glucosylated steviol glycoside, a hepta-glucosylated steviol glycoside, di-glucosylated kaurenoic acid, tri-glucosylated kaurenoic acid, di-glucosylated kaurenol, tri-glucosylated kaurenol, and isomers thereof.

[0051] Recombinant steviol glycoside-producing *Saccharomyces cerevisiae* (*S. cerevisiae*) strains are described in WO 2011/153378, WO 2013/022989, WO 2014/122227, and WO 2014/122328, each of which has been incorporated by reference herein in its entirety. See, also, Example 2. Methods of producing steviol glycosides in recombinant hosts, by whole cell bio-conversion, and *in vitro* are also described in WO 2011/153378, WO 2013/022989, WO 2014/122227, and WO 2014/122328.

[0052] In some embodiments, steviol glycosides and/or steviol glycoside precursors are produced *in vivo* through expression of one or more enzymes involved in the steviol glycoside biosynthetic pathway in a recombinant host. For example, a steviol-producing recombinant host expressing one or more of a gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide, a gene encoding an ent-copalyl diphosphate synthase (CDPS) polypeptide, a gene encoding a kaurene synthase (KS) polypeptide, a gene encoding a kaurene oxidase polypeptide (KO), a gene encoding a steviol synthase (KAH) polypeptide, a gene encoding a cytochrome P450 reductase (CPR) polypeptide, and a gene encoding a UGT polypeptide can produce a steviol glycoside and/or steviol glycoside precursors *in vivo*. See Example 2.

[0053] In some embodiments, a recombinant host comprises a nucleic acid encoding a UGT85C2 polypeptide, a nucleic acid encoding a UGT76G1 polypeptide, a nucleic acid encoding a UGT74G1 polypeptide, a nucleic acid encoding a UGT91D2 polypeptide, and/or a nucleic acid encoding a EUGT11 polypeptide. The skilled worker will appreciate that

expression of these genes may be necessary to produce a particular steviol glycoside but that one or more of these genes can be endogenous to the host provided that at least one (and in some embodiments, all) of these genes is a recombinant gene introduced into the microorganism. In a particular embodiment, a steviol-producing recombinant microorganism comprises exogenous nucleic acids encoding UGT85C2, UGT76G1, or UGT91D2 polypeptides. In another particular embodiment, a steviol-producing recombinant microorganism comprises exogenous nucleic acids encoding UGT85C2, UGT76G1, UGT74G1, and UGT91D2 polypeptides. In yet another particular embodiment, a steviol-producing recombinant microorganism comprises exogenous nucleic acids encoding UGT85C2, UGT76G1, UGT74G1, and EUGT11 polypeptides. In yet another particular embodiment, a steviol-producing recombinant microorganism comprises the exogenous nucleic acids encoding UGT85C2, UGT76G1, UGT74G1, UGT91D2 (including *inter alia* 91D2e, 91D2m, 91D2e-b, and functional homologs thereof), and EUGT11 polypeptides. See Example 2.

[0054] In certain embodiments, the steviol glycoside is RebA, RebB, RebD, and/or RebM. RebA can be synthesized in a steviol-producing recombinant microorganism expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2. RebB can be synthesized in a steviol-producing recombinant microorganism expressing UGT85C2, UGT76G1, and UGT91D2. RebD can be synthesized in a steviol-producing recombinant microorganism expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11. RebM can be synthesized in a steviol-producing recombinant microorganism expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11 (see Figure 1, Example 2).

[0055] In some embodiments, steviol glycosides and/or steviol glycoside precursors are produced through contact of a steviol glycoside precursor with one or more enzymes involved in the steviol glycoside pathway *in vitro*. For example, contacting steviol with a UGT polypeptide can result in production of a steviol glycoside *in vitro*. In some embodiments, a steviol glycoside precursor is produced through contact of an upstream steviol glycoside precursor with one or more enzymes involved in the steviol glycoside pathway *in vitro*. For example, contacting ent-kaurenoic acid with a KAH enzyme can result in production of steviol *in vitro*.

[0056] In some embodiments, a steviol glycoside or steviol glycoside precursor is produced by whole cell bioconversion. For whole cell bioconversion to occur, a host cell expressing one or more enzymes involved in the steviol glycoside pathway takes up and modifies a steviol glycoside precursor in the cell; following modification *in vivo*, a steviol glycoside remains in the cell and/or is excreted into the culture medium. For example, a host cell expressing a gene encoding a UGT polypeptide can take up steviol and glycosylate

steviol in the cell; following glycosylation *in vivo*, a steviol glycoside can be excreted into the culture medium. In some embodiments, the cell is permeabilized to take up a substrate to be modified or to excrete a modified product.

[0057] In some embodiments, a steviol glycoside or steviol glycoside precursor composition produced *in vivo*, *in vitro*, or by whole cell bioconversion comprises less contaminants than a stevia extract from, *inter alia*, a stevia plant. Contaminants include plant-derived compounds that contribute to off-flavors. Potential contaminants include pigments, lipids, proteins, phenolics, saccharides, spathulenol and other sesquiterpenes, labdane diterpenes, monoterpenes, decanoic acid, 8,11,14-eicosatrienoic acid, 2-methyloctadecane, pentacosane, octacosane, tetracosane, octadecanol, stigmasterol, β -sitosterol, α - and β -amyrin, lupeol, β -amryin acetate, pentacyclic triterpenes, centauredin, quercitin, epi- α -cadinol, carophyllenes and derivatives, beta-pinene, beta-sitosterol, and gibberellin.

[0058] As used herein, the terms "detectable amount," "detectable concentration," "measurable amount," and "measurable concentration" refer to a level of steviol glycosides measured in AUC, $\mu\text{M}/\text{OD}_{600}$, mg/L, μM , or mM. Steviol glycoside production (*i.e.*, total, supernatant, and/or intracellular steviol glycoside levels) can be detected and/or analyzed by techniques generally available to one skilled in the art, for example, but not limited to, liquid chromatography-mass spectrometry (LC-MS), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), ultraviolet-visible spectroscopy/spectrophotometry (UV-Vis), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR).

[0059] As used herein, the terms "or" and "and/or" is utilized to describe multiple components in combination or exclusive of one another. For example, "x, y, and/or z" can refer to "x" alone, "y" alone, "z" alone, "x, y, and z," "(x and y) or z," "x or (y and z)," or "x or y or z." In some embodiments, "and/or" is used to refer to the exogenous nucleic acids that a recombinant cell comprises, wherein a recombinant cell comprises one or more exogenous nucleic acids selected from a group. In some embodiments, "and/or" is used to refer to production of steviol glycosides and/or steviol glycoside precursors. In some embodiments, "and/or" is used to refer to production of steviol glycosides, wherein one or more steviol glycosides are produced. In some embodiments, "and/or" is used to refer to production of steviol glycosides, wherein one or more steviol glycosides are produced through one or more of the following steps: culturing a recombinant microorganism, synthesizing one or more steviol glycosides in a recombinant microorganism, and/or isolating one or more steviol glycosides.

Transporters and Transcription Factor Expression

[0060] This document describes reagents and methods that can be used to efficiently produce steviol glycoside compositions. Modification of transport systems in a recombinant host that are involved in transport of steviol glycosides into culture medium can allow more effective production of steviol glycosides in recombinant hosts.

[0061] As set forth herein, recombinant cells having modifications to cellular transport are capable of producing steviol. Recombinant hosts described herein can produce steviol and have altered expression of at least one endogenous transporter gene. Recombinant hosts described herein can produce steviol and have altered expression of a transcription factor that regulates expression of at least one endogenous transporter gene. Altering expression of endogenous transporter genes can be useful for increasing production of steviol and/or excretion of steviol into the culture medium.

[0062] As set forth herein, recombinant cells having modifications to cellular transport are capable of producing at least one steviol glycoside, including, but not limited to, RebA, RebB, RebD, and/or RebM. Recombinant hosts described herein can produce at least one steviol glycoside such as RebA, RebB, RebD, and/or RebM and have altered expression of at least one endogenous transporter gene. Recombinant hosts described herein can produce at least one steviol glycoside such as RebA, RebB, RebD, and/or RebM and have altered expression of a transcription factor that regulates expression of at least one endogenous transporter gene. Recombinant hosts described herein can produce at least one steviol glycoside such as RebA, RebB, RebD, and/or RebM and have altered expression of a plurality of endogenous transporter genes and/or of a plurality of transcription factor genes that regulate expression of a plurality of endogenous transporter genes. Altering expression of endogenous transporter genes and/or transcription factors regulating expression of at least one transporter gene can be useful for increasing production of steviol glycosides and/or excretion of steviol glycosides into the culture medium.

[0063] Recombinant hosts disclosed herein can include one or more biosynthesis genes, such as one or more genes encoding a sucrose transporter and a sucrose synthase; a gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide; a gene encoding an ent-copalyl diphosphate synthase (CDPS) polypeptide; a gene encoding a kaurene synthase (KS) polypeptide; a gene encoding a kaurene oxidase (KO) polypeptide; a gene encoding a steviol synthase (KAH) polypeptide; a gene encoding a cytochrome P450 reductase (CPR) polypeptide; a gene encoding a UGT85C2 polypeptide; a gene encoding a

UGT76G1 polypeptide; a gene encoding a UGT74G1 polypeptide; a gene encoding a a UGT91D2 functional homolog; and/or a gene encoding a EUGT11 polypeptide; wherein expression of one or more of these genes results in production of steviol glycosides such as RebA, RebB, RebD, and/or RebM.

[0064] As used herein, the terms “transport of a steviol glycoside,” “steviol glycoside transport,” “excretion of a steviol glycoside,” and “steviol glycoside excretion” can be used interchangeably.

[0065] As used herein, the term “transporter” (also referred to as a membrane transport protein) refers to a membrane protein involved in the movement of small molecules, macromolecules (such as carbohydrates), and ions across a biological membrane. Transporters span the membrane in which they are localized and across which they transport substances. Transporter proteins can assist in the movement (*i.e.*, transport or excretion) of a substance from the intracellular space to the culture medium. Transporters are known to function as passive transport systems, carrying molecules down their concentration gradient, or as active transport systems, using energy to carry molecules uphill against their concentration gradient. Active transport is mediated by carriers which couple transport directly to the use of energy derived from hydrolysis of an ATP molecule or by carriers which make use of a pre-established electrochemical ion gradient to drive co-transport of the nutrient molecule and a co-transported ion. The latter category comprises symporters and antiporters, which carry the ion in the same or opposite direction, respectively, as the transported substrate.

[0066] Transport proteins have been classified according to various criteria at the Transporter Classification Database (on the world wide web at tcdb.org). See, Saier Jr. *et al.*, Nucl. Acids Res., 42(1):D251-258 (2014). Non-limiting examples thereof include, among others, the family of Multiple Drug Resistance (MDR) plasma membrane transporters that is thought to be ubiquitous among living organisms. The MDR transporter superfamily can be further subdivided according to the mode of operation by which the substrate is transported from one side of the membrane to the other. Transporters can operate to move substances across membranes in response to chemiosmotic ion gradients or by active transport. ATP-binding cassette transporters (ABC transporters) are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out translocation of various substrates across membranes. They can transport a wide variety of substrates across the plasma membrane and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Particular non-limiting examples of endogenous ABC transporter genes include PDR5, YDR061W, PDR15, SNQ2, YOR1, YOL075C, MDL2, ADP1, CAF16, VMR1

and STE6 (or a functional homolog thereof). In some aspects, ABC transporters transport steviol glycosides.

[0067] A second group of MDRs is further subdivided based on the nature of the chemiosmotic gradient that facilitates the transport. Saier, Jr. *et al.*, J. Mol. Microbiol. Biotechnol. 1:257-279 (1999). In some aspects, MDR transporters transport steviol glycosides.

[0068] Another transporter family, the Major Facilitator Superfamily (MFS) transporters are monomeric polypeptides that can transport small solutes in response to proton gradients. The MFS transporter family is sometimes referred to as the uniporter-symporter-antiporter family. MFS transporters function in, *inter alia*, in sugar uptake and drug efflux systems. MFS transporters typically comprise conserved MFS-specific motifs. Non-limiting examples of endogenous MFS transporter genes include DTR1, SEO1, YBR241C, VBA3, FEN2, SNF3, STL1, HXT10, AZR1, MPH3, VBA5, GEX2, SNQ1, AQR1, MCH1, MCH5, ATG22, HXT15, MPH2, ITR1, SIT1, VPS73, HXT5, QDR1, QDR2, QDR3, SOA1, HXT9, YMR279C, YIL166C, HOL1, ENB1, TPO4 and FLR1 (or a functional homolog thereof). In some aspects, MFS transporters transport steviol glycosides.

[0069] Other transporter families include the SMR (small multidrug resistant) family, RND (Resistance-Nodulation-Cell Division) family, and the MATE (multidrug and toxic compound extrusion) family. The SMR family members are integral membrane proteins characterized by four alpha-helical transmembrane strands that confer resistance to a broad range of antiseptics, lipophilic quaternary ammonium compounds (QAC), and aminoglycoside resistance in bacteria. See, Bay & Turner, 2009, BMC Evol Biol., 9:140. In some aspects, SMR transporters transport steviol glycosides.

[0070] The MATE family members comprise 12 transmembrane (TM) domains. Members of the MATE family have been identified in prokaryotes, yeast such as *S. cerevisiae* and *Schizosaccharomyces pombe*, and plants. See Diener *et al.*, 2001, Plant Cell. 13(7):1625-8. The MATE family members are sodium or proton antiporters. In some aspects, MATE transporters transport steviol glycosides.

[0071] Additional transporter families include the amino acid/auxin permease (AAP) family (for example, YKL146W/AVT3, YBL089W/AVT5, YER119C/AVT6 and YIL088C/AVT7), the ATPase family (for example, YBL099W/ATP1, YDL185W/VMA1, YLR447C/VMA6, YOL077W/ATP19, YPL078C/ATP4, YEL027W/VMA3, YKL016C/ATP7, and YOR332W/VMA4), the sulfate permease (SulP) family (for example, YBR294W/SUL1, YGR125W and YPR003C), the lysosomal cystine transporter (LCT) family (for example, YCR075C/ERS1), the Ca²⁺:cation antiporter (CaCA) family (for example, YDL128W/VCX1

and YJR106W/ECM27), the amino acid-polyamine-organocation (APC) superfamily (for example, YDL210W/UGA4, YOL020W/TAT2, YPL274W/SAM3, YNL268W/LYP1, YHL036W/MUP3, YKR039W/GAP1 and YOR348C/PUT4), multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) (for example, YDR338C), the ZRT/IRT-like protein (ZIP) metal transporter family (for example, YGL225W/ZRT1 and YOR079C/ATX2), the mitochondrial protein translocase (MPT) family (for example, YGR181W/TIM13, YNL070W/TOM7, YNL121C/TOM70, the voltage-gated ion channel (VIC) family (for example, YGR217W/CCH1 and YJL093C/TOK1), the monovalent cation:proton antiporter-2 (CPA2) family (for example, YJL094C/KHA1), the ThrE family of putative transmembrane amino acid efflux transporters (for example, YJL108C/PRM10), the oligopeptide transporter (OPT) family (for example, YJL212C/OPT1 and YGL114W), the K⁺ transporter (Trk) family (for example, TKR050W/TRK2), the bile acid:Na symporter (BASS) family (for example, YMR034C), the drug/metabolite transporter (DMT) superfamily (for example, YMR253C, YML038C/ YMD8, and YOR307C/SLY41), the mitochondrial carrier (MC) family (for example, YMR056C/AAC1, YNL083W/SAL1, YOR130C/ORT1, YOR222W/ODC2, YPR011C, YPR058W/YMC1, YPR128C/ANT1, YEL006W/YEA6, YER053C/PIC2, YFR045W, YGR257C/MTM1, YHR002W/LEU5, YIL006W/YIA6, YJL133W/MRS3, YKL120W/OAC1, YMR166C, YNL003C/PET8 and YOR100C/CRC1), the auxin efflux carrier (AEC) family (for example, YNL095C, YOR092W/ECM3 and YBR287W), the ammonia channel transporter (Amt) family (for example, YNL142W/MEP2), the metal ion (Mn²⁺-iron) transporter (Nramp) family (for example, YOL122C/SMF1), the transient receptor potential Ca²⁺ channel (TRP-CC) family (for example, YOR087W/YVC1), the arsenical resistance-3 (ACR3) family (for example, YPR201W/ARR3), the nucleobase:cation symporter-1 (NCS1) family (for example, YBR021W/FUR4), the inorganic phosphate transporter (PiT) family (for example, YBR296C/PHO89), the arsenite-antimonite (ArsAB) efflux family (for example, YDL100C/GET3), the IISP family of transporters, the glycerol uptake (GUP) family (for example, YGL084C/GUP1), the metal ion transport (MIT) family (for example, YKL064W/MNR2, YKL050C and YOR334W/MRS2), the copper transport (Ctr) family (for example, YLR411W/CTR3) and the cation diffusion facilitator (CDF) family (for example, YOR316C/COT1). Particular members of any of these transporter families are included within the scope of the disclosed invention to the extent that altered expression in a cell capable of producing steviol glycoside increases production of said steviol glycoside from the cell; exemplary members are disclosed above and in Tables 5, 6, and 14.

[0072] As used herein, the term “transcription factor” refers to a DNA-binding protein that regulates gene expression. Preferably, the transcription factor regulates expression of at least one transporter gene.

[0073] Methods for identifying a gene affecting production or transport of steviol glycosides and steviol glycoside pathway intermediates are disclosed herein. Such methods can involve inactivating at least one endogenous transporter gene or modifying expression of at least one transporter gene. Typically, a library of mutant microorganisms is prepared, each mutant in the library having a different endogenous transporter gene inactivated. Methods of inactivating genes and determining their effect in a microorganisms are known to a person having ordinary skill in the art; additional methods are disclosed in WO 2014/122328, the disclosure of which is incorporated by reference in its entirety. The mutant microorganisms comprising one or more steviol glycoside pathway genes are cultured in a medium under conditions in which steviol or a steviol glycoside is synthesized, and the amount of total, supernatant, and/or intracellular steviol glycosides produced by the microorganism is measured (e.g., using LC-MS) as described herein.

[0074] The disclosure is directed to recombinant host cells in which expression of endogenous transporter or transcription factor genes is modified. In some embodiments, the transporter or transcription factor gene is endogenous to *S. cerevisiae*, including, but not limited to *S. cerevisiae* strain S288C. In some embodiments, expression of an endogenous transporter or transcription factor can be modified by replacing the endogenous promoter with a different promoter that results in increased expression of the transporter protein (e.g., at least a 5% increase in expression, such as at least a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, 100%, 200% increase or more in expression). For example, an endogenous promoter can be replaced with a constitutive or inducible promoter that results in increased expression of the transporter. Homologous recombination can be used to replace the promoter of an endogenous gene with a different promoter that results in increased expression of the transporter. In other embodiments, the inducible or constitutive promoter and endogenous transporter or transcription factor can be integrated into another locus of the genome using homologous recombination. In other embodiments, the transporter or transcription factor gene can be introduced into a microorganism using exogenous plasmids with a promoter that results in overexpression of the transporter or transcription factor in the microorganism. In yet another embodiment, the exogenous plasmids may also comprise multiple copies of the transporter or transcription factor gene. In a further embodiment, the endogenous transporter or transcription factor can be induced to be overexpressed using native mechanisms to the recombinant microorganism (e.g. heat shock, stress, heavy metal, or antibiotic exposure). In yet a further embodiment, the activity of an endogenous gene product is enhanced or increased (for example, by mutation). In yet another embodiment, a homologous or orthologous gene of an endogenous yeast transporter or transcription factor gene is overexpressed.

[0075] In certain other embodiments, modified expression of a target gene in a recombinant microorganism comprises overexpressing a transporter gene and/or a transcription factor gene involved in expression of said transporter gene. In yet other embodiments, a plurality of endogenous transporter genes or transcription factor genes is overexpressed in said recombinant microorganism.

[0076] Modification of transcription factor expression can be used to increase transporter expression. For example, yeast transcription factor PDR1 regulates expression of the genes encoding ABC transporters PDR5, SNQ2 and YOR1. Therefore, in some embodiments, promoters for the endogenous PDR1 locus can be replaced with a different promoter that results in increased expression of the transcription factors, which can increase production of endogenous transporters.

[0077] In some embodiments, the transporter gene or transcription factor gene is (using Uniprot Ordered Locus Name for each): YAL067C, YBL089W, YBL099W, YBR008C, YBR021W, YBR043C, YBR180W, YBR241C, YBR287W, YBR294W, YBR295W, YBR296C, YCL038C, YCL069W, YCR011C, YCR028C, YCR075C, YDL054C, YDL100C, YDL128W, YDL185W, YDL194W, YDL210W, YDL245C, YDL247W, YDR011W, YDR061W, YDR093W, YDR292C, YDR338C, YDR406W, YDR497C, YDR536W, YEL006W, YEL027W, YEL031W, YEL065W, YER019C-A, YER053C, YER119C, YER166W, YFL011W, YFL028C, YFR045W, YGL006W, YGL013C, YGL084C, YGL104C, YGL114W, YGL167C, YGL255W, YGR125W, YGR181W, YGR217W, YGR224W, YGR257C, YGR281W, YHL016C, YHL035C, YHL036W, YHR002W, YHR096C, YIL006W, YIL088C, YIL120W, YIL121W, YIL166C, YJL093C, YJL094C, YJL108C, YJL133W, YJL212C, YJL219W, YJR106W, YJR160C, YKL016C, YKL050C, YKL064W, YKL120W, YKL146W, YKL209C, YKR039W, YKR050W, YKR105C, YKR106W, YLR411W, YLR447C, YML038C, YML116W, YMR034C, YMR056C, YMR166C, YMR253C, YMR279C, YNL003C, YNL065W, YNL070W, YNL083W, YNL095C, YNL121C, YNL142W, YNL268W, YNR055C, YOL020W, YOL075C, YOL077W-A, YOL122C, YOL158C, YOR079C, YOR087W, YOR092W, YOR100C, YOR130C, YOR153W, YOR222W, YOR271C, YOR273C, YOR291W, YOR306C, YOR307C, YOR316C, YOR332W, YOR334W, YOR348C, YPL036W, YPL078C, YPL270W, YPL274W, YPR003C, YPR011C, YPR058W, YPR128C, and/or YPR201W. SEQ ID NOs, Uniprot Accession Numbers, and gene names for each Ordered Locus can be found in Tables 5, 6, and 14. In some embodiments, the above transporter genes and transcription factor genes regulate excretion of steviol glycosides.

[0078] In some embodiments, deletion in a steviol glycoside-producing strain of YDL128W (SEQ ID NO:22), YDL194W (SEQ ID NO:24), YDL210W (SEQ ID NO:25), YDR536W (SEQ ID NO:30), YFL011W (SEQ ID NO:33), YGL006W (SEQ ID NO:34),

YGL013C (SEQ ID NO:35), YGL255W (SEQ ID NO:36), YGR181W (SEQ ID NO:38), YGR217W (SEQ ID NO:39), YHL016C (SEQ ID NO:42), YIL088C (SEQ ID NO:43), YJL094C (SEQ ID NO:45), YJR106W (SEQ ID NO:48), YKR050W (SEQ ID NO:51), YNL065W (SEQ ID NO:59), YNL083W (SEQ ID NO:61), YNL121C (SEQ ID NO:63), YNL142W (SEQ ID NO:64), YOR291W (SEQ ID NO:74), YOR306C (SEQ ID NO:75), YOR334W (SEQ ID NO:77), YPL270W (SEQ ID NO:79), YPR011C (SEQ ID NO:82), YPR128C (SEQ ID NO:84) results in a measurable decrease of RebD excreted into the culture medium, indicating that each plays a role in RebD excretion. See Example 3 and Tables 7-10.

[0079] In some embodiments, deletion in a steviol glycoside-producing strain of YBR180W (SEQ ID NO:13), YAL067C (SEQ ID NO:14), YBR241C (SEQ ID NO:17), YCL069W (SEQ ID NO:19), YCR075C (SEQ ID NO:21), YDL128W (SEQ ID NO:22), YDL194W (SEQ ID NO:24), YDR093W (SEQ ID NO:27), YDR338C (SEQ ID NO:28), YDR406W (SEQ ID NO:29), YER166W (SEQ ID NO:32), YFL011W (SEQ ID NO:33), YGL006W (SEQ ID NO:34), YGL013C (SEQ ID NO:35), YGL255W (SEQ ID NO:36), YGR217W (SEQ ID NO:39), YHL016C (SEQ ID NO:42), YJL094C (SEQ ID NO:45), YJL212C (SEQ ID NO:47), YJR106W (SEQ ID NO:48), YJR160C (SEQ ID NO:49), YKR050W (SEQ ID NO:51), YKR106W (SEQ ID NO:53), YML116W (SEQ ID NO:55), YMR034C (SEQ ID NO:56), YMR056C (SEQ ID NO:57), YMR253C (SEQ ID NO:58), YNL070W (SEQ ID NO:60), YNL083W (SEQ ID NO:61), YNL095C (SEQ ID NO:62), YNL121C (SEQ ID NO:63), YOL075C (SEQ ID NO:66), YOL122C (SEQ ID NO:68), YOR087W (SEQ ID NO:70), YOR222W (SEQ ID NO:73), YOR291W (SEQ ID NO:74), YOR306C (SEQ ID NO:75), YPL274W (SEQ ID NO:80), YPR003C (SEQ ID NO:81), YPR011C (SEQ ID NO:82), or YPR201W (SEQ ID NO:85) results in a measurable decrease of RebM, indicating that each plays a role in RebM excretion. See Example 3 and Tables 7-10.

[0080] In some embodiments, overexpression of YGR181W (SEQ ID NO:38) or YDR061W (SEQ ID NO:26) improves RebD and RebM transport into the culture medium by approximately 2-fold (~400-500 mg/L of supernatant RebD and RebM in YGR181W (SEQ ID NO:38) and YDR061W (SEQ ID NO:26) overexpression strains versus ~250 mg/L of supernatant RebD and RebM in a control steviol glycoside-producing strain). See Example 4, Figure 2, and Figure 3.

[0081] In some embodiments, overexpression of a transporter of Table 11 increases excretion of RebA, RebB, RebD, and/or RebM by at least 20%. In some embodiments, overexpression of a transporter of Table 12 increases production of RebA, RebB, RebD, and/or RebM by at least 40%. See Example 5.

[0082] In some embodiments, a transporter gene is integrated into the genome of a steviol glycoside-producing host. In some embodiments, the integrated transporter is YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), YJL093C (SEQ ID NO:44), YJR106W (SEQ ID NO:48), YMR166C (SEQ ID NO:132), YIL166C (SEQ ID NO:121), YKL120W (SEQ ID NO:126), YDL054C (SEQ ID NO:94), YDL128W (SEQ ID NO:22), YDR536W (SEQ ID NO:30), YGL167C (SEQ ID NO:112), YKL146W (SEQ ID NO:127), YKR039W (SEQ ID NO:129), YOL122C (SEQ ID NO:68), or YPR011C (SEQ ID NO:82). In some embodiments, integration of YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), YJL093C (SEQ ID NO:44), YJR106W (SEQ ID NO:48), YKL120W (SEQ ID NO:126), or YMR166C (SEQ ID NO:132) improves excretion and/or total production of 13-SMG. In some embodiments, integration of YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), or YMR166C (SEQ ID NO:132) improves excretion and/or total production of RebA. In some embodiments, integration of YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), or YMR166C (SEQ ID NO:132) improves excretion and/or total production of RebB. In some embodiments, integration of YBR043C of SEQ ID NO:88, YEL027W of SEQ ID NO:102, YJL093C of SEQ ID NO:44, YJR106W of SEQ ID NO:48, and YMR166C of SEQ ID NO:132 improves excretion and/or total production of RebD, and YBR043C of SEQ ID NO:88, YEL027W of SEQ ID NO:102, YIL166C (SEQ ID NO:121), YJL093C of SEQ ID NO:44, YJR106W of SEQ ID NO:48, and YMR166C of SEQ ID NO:132 improves excretion and/or total production of RebM, as measured by an increase in RebD and RebM levels in the supernatant compared to a control steviol glycoside-producing strain. See Example 6.

[0083] In some embodiments, steviol glycoside-producing *S. cerevisiae* strains overexpressing YJL093C (SEQ ID NO:44) or YBR043C (SEQ ID NO:88) produce higher levels of RebD + RebM, compared to a steviol glycoside-producing *S. cerevisiae* strain that does not overexpress YJL093C or YBR043C. See Example 7.

[0084] In some embodiments, a transporter that is knocked out can also have specificity for transport of larger molecular weight steviol glycosides (for example, RebD and the knockout of YGR181W of SEQ ID NO:38 or YOR291W of SEQ ID NO:74), and therefore, can be useful to overexpress in strains where transport of RebD into the culture medium is desired. With appropriate balancing of the rate of glycosylation activity through expression of pathway UGTs, smaller molecular weight steviol glycosides are further glycosylated before they are transported into the culture medium. For example, higher expression levels of a UGT76G1 and UGT91D2e and/or EUGT11, as compared to the UGT74G1 and UGT85C2 enzymes, can prevent accumulation of the steviol monoglucosides that are transported more readily. If the UGT activity level is higher (so the glycosylation rate is

faster) than the rate of transport, then greater amounts of larger molecular weight steviol glycosides will be produced.

Steviol and Steviol Glycoside Biosynthesis Nucleic Acids

[0085] A recombinant gene encoding a polypeptide described herein comprises the coding sequence for that polypeptide, operably linked in sense orientation to one or more regulatory regions suitable for expressing the polypeptide. Because many microorganisms are capable of expressing multiple gene products from a polycistronic mRNA, multiple polypeptides can be expressed under the control of a single regulatory region for those microorganisms, if desired. A coding sequence and a regulatory region are considered to be operably linked when the regulatory region and coding sequence are positioned so that the regulatory region is effective for regulating transcription or translation of the sequence. Typically, the translation initiation site of the translational reading frame of the coding sequence is positioned between one and about fifty nucleotides downstream of the regulatory region for a monocistronic gene.

[0086] In many cases, the coding sequence for a polypeptide described herein is identified in a species other than the recombinant host, *i.e.*, is a heterologous nucleic acid. Thus, if the recombinant host is a microorganism, the coding sequence can be from other prokaryotic or eukaryotic microorganisms, from plants or from animals. In some case, however, the coding sequence is a sequence that is native to the host and is being reintroduced into that organism. A native sequence can often be distinguished from the naturally occurring sequence by the presence of non-natural sequences linked to the exogenous nucleic acid, *e.g.*, non-native regulatory sequences flanking a native sequence in a recombinant nucleic acid construct. In addition, stably transformed exogenous nucleic acids typically are integrated at positions other than the position where the native sequence is found. "Regulatory region" refers to a nucleic acid having nucleotide sequences that influence transcription or translation initiation and rate, and stability and/or mobility of a transcription or translation product. Regulatory regions include, without limitation, promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, protein binding sequences, 5' and 3' untranslated regions (UTRs), transcriptional start sites, termination sequences, polyadenylation sequences, introns, and combinations thereof. A regulatory region typically comprises at least a core (basal) promoter. A regulatory region also may include at least one control element, such as an enhancer sequence, an upstream element or an upstream activation region (UAR). A regulatory region is operably linked to a coding sequence by positioning the regulatory region and the coding

sequence so that the regulatory region is effective for regulating transcription or translation of the sequence. For example, to operably link a coding sequence and a promoter sequence, the translation initiation site of the translational reading frame of the coding sequence is typically positioned between one and about fifty nucleotides downstream of the promoter. A regulatory region can, however, be positioned as much as about 5,000 nucleotides upstream of the translation initiation site, or about 2,000 nucleotides upstream of the transcription start site.

[0087] The choice of regulatory regions to be included depends upon several factors, including, but not limited to, efficiency, selectability, inducibility, desired expression level, and preferential expression during certain culture stages. It is a routine matter for one of skill in the art to modulate the expression of a coding sequence by appropriately selecting and positioning regulatory regions relative to the coding sequence. It will be understood that more than one regulatory region may be present, *e.g.*, introns, enhancers, upstream activation regions, transcription terminators, and inducible elements.

[0088] One or more genes can be combined in a recombinant nucleic acid construct in “modules” useful for a discrete aspect of steviol and/or steviol glycoside production. Combining a plurality of genes in a module, particularly a polycistronic module, facilitates the use of the module in a variety of species. For example, a steviol biosynthesis gene cluster, or a UGT gene cluster, can be combined in a polycistronic module such that, after insertion of a suitable regulatory region, the module can be introduced into a wide variety of species. As another example, a UGT gene cluster can be combined such that each UGT coding sequence is operably linked to a separate regulatory region, to form a UGT module. Such a module can be used in those species for which monocistronic expression is necessary or desirable. In addition to genes useful for steviol or steviol glycoside production, a recombinant construct typically also comprises an origin of replication, and one or more selectable markers for maintenance of the construct in appropriate species.

[0089] It will be appreciated that because of the degeneracy of the genetic code, a number of nucleic acids can encode a particular polypeptide; *i.e.*, for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. Thus, codons in the coding sequence for a given polypeptide can be modified such that optimal expression in a particular host is obtained, using appropriate codon bias tables for that host (*e.g.*, microorganism). As isolated nucleic acids, these modified sequences can exist as purified molecules and can be incorporated into a vector or a virus for use in constructing modules for recombinant nucleic acid constructs.

[0090] In some cases, it is desirable to inhibit one or more functions of an endogenous polypeptide in order to divert metabolic intermediates towards steviol or steviol glycoside biosynthesis. For example, it may be desirable to downregulate synthesis of sterols in a strain in order to further increase steviol or steviol glycoside production, e.g., by downregulating squalene epoxidase. As another example, it may be desirable to inhibit degradative functions of certain endogenous gene products, e.g., glycohydrolases that remove glucose moieties from secondary metabolites or phosphatases as discussed herein. As another example, expression of membrane transporters involved in transport of steviol glycosides can be activated, such that transportation of steviol glycosides is increased. Such regulation can be beneficial in that transportation of steviol glycosides can be increased for a desired period of time during culture of the microorganism, thereby increasing the yield of glycoside product(s) at harvest. In such cases, a nucleic acid that overexpresses the polypeptide or gene product may be included in a recombinant construct that is transformed into the strain. Alternatively, mutagenesis can be used to generate mutants in genes for which it is desired to increase or enhance function.

Recombinant Hosts

[0091] Recombinant hosts can be used to express polypeptides for the producing steviol glycosides, including mammalian, insect, plant, and algal cells. A number of prokaryotes and eukaryotes are also suitable for use in constructing the recombinant microorganisms described herein, e.g., gram-negative bacteria, yeast, and fungi. A species and strain selected for use as a steviol glycoside production strain is first analyzed to determine which production genes are endogenous to the strain and which genes are not present. Genes for which an endogenous counterpart is not present in the strain are advantageously assembled in one or more recombinant constructs, which are then transformed into the strain in order to supply the missing function(s).

[0092] Typically, the recombinant microorganism is grown in a fermenter at a defined temperature(s) for a desired period of time. The constructed and genetically engineered microorganisms provided by the invention can be cultivated using conventional fermentation processes, including, *inter alia*, chemostat, batch, fed-batch cultivations, semi-continuous fermentations such as draw and fill, continuous perfusion fermentation, and continuous perfusion cell culture. Depending on the particular microorganism used in the method, other recombinant genes such as isopentenyl biosynthesis genes and terpene synthase and cyclase genes may also be present and expressed. Levels of substrates and intermediates, e.g., isopentenyl diphosphate, dimethylallyl diphosphate, GGPP, kaurene and kaurenoic

acid, can be determined by extracting samples from culture media for analysis according to published methods.

[0093] Carbon sources of use in the instant method include any molecule that can be metabolized by the recombinant host cell to facilitate growth and/or production of the steviol glycosides. Examples of suitable carbon sources include, but are not limited to, sucrose (e.g., as found in molasses), fructose, xylose, ethanol, glycerol, glucose, cellulose, starch, cellobiose or other glucose-comprising polymer. In embodiments employing yeast as a host, for example, carbon sources such as sucrose, fructose, xylose, ethanol, glycerol, and glucose are suitable. The carbon source can be provided to the host organism throughout the cultivation period or alternatively, the organism can be grown for a period of time in the presence of another energy source, e.g., protein, and then provided with a source of carbon only during the fed-batch phase.

[0094] After the recombinant microorganism has been grown in culture for the desired period of time, steviol and/or one or more steviol glycosides can then be recovered from the culture using various techniques known in the art. In some embodiments, a permeabilizing agent can be added to aid the feedstock entering into the host and product getting out. For example, a crude lysate of the cultured microorganism can be centrifuged to obtain a supernatant. The resulting supernatant can then be applied to a chromatography column, e.g., a C-18 column, and washed with water to remove hydrophilic compounds, followed by elution of the compound(s) of interest with a solvent such as methanol. The compound(s) can then be further purified by preparative HPLC. See *also*, WO 2009/140394.

[0095] It will be appreciated that the various genes and modules discussed herein can be present in two or more recombinant hosts rather than a single host. When a plurality of recombinant hosts is used, they can be grown in a mixed culture to produce steviol and/or steviol glycosides.

[0096] Alternatively, the two or more hosts each can be grown in a separate culture medium and the product of the first culture medium, e.g., steviol, can be introduced into second culture medium to be converted into a subsequent intermediate, or into an end product such as, for example, RebA. The product produced by the second, or final host is then recovered. It will also be appreciated that in some embodiments, a recombinant host is grown using nutrient sources other than a culture medium and utilizing a system other than a fermenter.

[0097] Exemplary prokaryotic and eukaryotic species are described in more detail below. However, it will be appreciated that other species can be suitable. For example, suitable species can be in a genus such as *Agaricus*, *Aspergillus*, *Bacillus*, *Candida*,

Corynebacterium, *Eremothecium*, *Escherichia*, *Fusarium*/*Gibberella*, *Kluyveromyces*, *Laetiporus*, *Lentinus*, *Phaffia*, *Phanerochaete*, *Pichia*, *Physcomitrella*, *Rhodoturula*, *Saccharomyces*, *Schizosaccharomyces*, *Sphaceloma*, *Xanthophyllomyces* or *Yarrowia*. Exemplary species from such genera include *Lentinus tigrinus*, *Laetiporus sulphureus*, *Phanerochaete chrysosporium*, *Pichia pastoris*, *Cyberlindnera jadinii*, *Physcomitrella patens*, *Rhodoturula glutinis*, *Rhodoturula mucilaginosa*, *Phaffia rhodozyma*, *Xanthophyllomyces dendrorhous*, *Fusarium fujikuroi*/*Gibberella fujikuroi*, *Candida utilis*, *Candida glabrata*, *Candida albicans*, and *Yarrowia lipolytica*.

[0098] In some embodiments, a microorganism can be a prokaryote such as *Escherichia coli*.

[0099] In some embodiments, a microorganism can be an Ascomycete such as *Gibberella fujikuroi*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Aspergillus niger*, *Yarrowia lipolytica*, *Ashbya gossypii*, or *S. cerevisiae*.

[00100] In some embodiments, a microorganism can be an algal cell such as *Blakeslea trispora*, *Dunaliella salina*, *Haematococcus pluvialis*, *Chlorella* sp., *Undaria pinnatifida*, *Sargassum*, *Laminaria japonica*, *Scenedesmus almeriensis* species.

[00101] In some embodiments, a microorganism can be a cyanobacterial cell such as *Blakeslea trispora*, *Dunaliella salina*, *Haematococcus pluvialis*, *Chlorella* sp., *Undaria pinnatifida*, *Sargassum*, *Laminaria japonica*, *Scenedesmus almeriensis*.

Saccharomyces spp.

[00102] *Saccharomyces* is a widely used chassis organism in synthetic biology, and can be used as the recombinant microorganism platform. For example, there are libraries of mutants, plasmids, detailed computer models of metabolism and other information available for *S. cerevisiae*, allowing for rational design of various modules to enhance product yield. Methods are known for making recombinant microorganisms.

Aspergillus spp.

[00103] *Aspergillus* species such as *A. oryzae*, *A. niger* and *A. sojae* are widely used microorganisms in food production and can also be used as the recombinant microorganism platform. Nucleotide sequences are available for genomes of *A. nidulans*, *A. fumigatus*, *A. oryzae*, *A. clavatus*, *A. flavus*, *A. niger*, and *A. terreus*, allowing rational design and modification of endogenous pathways to enhance flux and increase product yield. Metabolic models have been developed for *Aspergillus*, as well as transcriptomic studies and proteomics studies. *A. niger* is cultured for the industrial production of a number of food

ingredients such as citric acid and gluconic acid, and thus species such as *A. niger* are generally suitable for producing steviol glycosides.

E. coli

[00104] *E. coli*, another widely used platform organism in synthetic biology, can also be used as the recombinant microorganism platform. Similar to *Saccharomyces*, there are libraries of mutants, plasmids, detailed computer models of metabolism and other information available for *E. coli*, allowing for rational design of various modules to enhance product yield. Methods similar to those described above for *Saccharomyces* can be used to make recombinant *E. coli* microorganisms.

[00105] *Agaricus, Gibberella, and Phanerochaete spp.*

[00106] *Agaricus, Gibberella, and Phanerochaete spp.* can be useful because they are known to produce large amounts of isoprenoids in culture. Thus, the terpene precursors for producing large amounts of steviol glycosides are already produced by endogenous genes. Thus, modules comprising recombinant genes for steviol glycoside biosynthesis polypeptides can be introduced into species from such genera without the necessity of introducing mevalonate or MEP pathway genes.

Arxula adeninivorans (Blastobotrys adeninivorans)

[00107] *Arxula adeninivorans* is dimorphic yeast (it grows as budding yeast like the baker's yeast up to a temperature of 42°C, above this threshold it grows in a filamentous form) with unusual biochemical characteristics. It can grow on a wide range of substrates and can assimilate nitrate. It has successfully been applied to the generation of strains that can produce natural plastics or the development of a biosensor for estrogens in environmental samples.

Yarrowia lipolytica

[00108] *Yarrowia lipolytica* is dimorphic yeast (see *Arxula adeninivorans*) and belongs to the family Hemiascomycetes. The entire genome of *Yarrowia lipolytica* is known. *Yarrowia* species is aerobic and considered to be non-pathogenic. *Yarrowia* is efficient in using hydrophobic substrates (e.g. alkanes, fatty acids, oils) and can grow on sugars. It has a high potential for industrial applications and is an oleaginous microorganism. *Yarrowia lipolytica* can accumulate lipid content to approximately 40% of its dry cell weight and is a model organism for lipid accumulation and remobilization. See e.g., Nicaud, 2012, *Yeast* 29(10):409-18; Beopoulos *et al.*, 2009, *Biochimie* 91(6):692-6; Bankar *et al.*, 2009, *Appl Microbiol Biotechnol.* 84(5):847-65.

Rhodotorula sp.

[00109] *Rhodotorula* is unicellular, pigmented yeast. The oleaginous red yeast, *Rhodotorula glutinis*, has been shown to produce lipids and carotenoids from crude glycerol (Saenge *et al.*, 2011, *Process Biochemistry* 46(1):210-8). *Rhodotorula toruloides* strains have been shown to be an efficient fed-batch fermentation system for improved biomass and lipid productivity (Li *et al.*, 2007, *Enzyme and Microbial Technology* 41:312-7).

Rhodospiridium toruloides

[00110] *Rhodospiridium toruloides* is oleaginous yeast and useful for engineering lipid-production pathways (See e.g. Zhu *et al.*, 2013, *Nature Commun.* 3:1112; Ageitos *et al.*, 2011, *Applied Microbiology and Biotechnology* 90(4):1219-27).

Candida boidinii

[00111] *Candida boidinii* is methylotrophic yeast (it can grow on methanol). Like other methylotrophic species such as *Hansenula polymorpha* and *Pichia pastoris*, it provides an excellent platform for producing heterologous proteins. Yields in a multigram range of a secreted foreign protein have been reported. A computational method, IPRO, recently predicted mutations that experimentally switched the cofactor specificity of *Candida boidinii* xylose reductase from NADPH to NADH. See, e.g., Mattanovich *et al.*, 2012, *Methods Mol Biol.* 824:329-58; Khoury *et al.*, 2009, *Protein Sci.* 18(10):2125-38.

Hansenula polymorpha (*Pichia angusta*)

[00112] *Hansenula polymorpha* is methylotrophic yeast (see *Candida boidinii*). It can furthermore grow on a wide range of other substrates; it is thermo-tolerant and can assimilate nitrate (see also *Kluyveromyces lactis*). It has been applied to producing hepatitis B vaccines, insulin and interferon alpha-2a for the treatment of hepatitis C, furthermore to a range of technical enzymes. See, e.g., Xu *et al.*, 2014, *Virol Sin.* 29(6):403-9.

Kluyveromyces lactis

[00113] *Kluyveromyces lactis* is yeast regularly applied to the production of kefir. It can grow on several sugars, most importantly on lactose which is present in milk and whey. It has successfully been applied among others for producing chymosin (an enzyme that is usually present in the stomach of calves) for producing cheese. Production takes place in fermenters on a 40,000 L scale. See, e.g., van Ooyen *et al.*, 2006, *FEMS Yeast Res.* 6(3):381-92.

Pichia pastoris

[00114] *Pichia pastoris* is methylotrophic yeast (see *Candida boidinii* and *Hansenula polymorpha*). It provides an efficient platform for producing foreign proteins. Platform

elements are available as a kit and it is worldwide used in academia for producing proteins. Strains have been engineered that can produce complex human N-glycan (yeast glycans are similar but not identical to those found in humans). See, e.g., Piirainen *et al.*, 2014, *N Biotechnol.* 31(6):532-7.

Physcomitrella spp.

[00115] *Physcomitrella* mosses, when grown in suspension culture, have characteristics similar to yeast or other fungal cultures. This genera can be used for producing plant secondary metabolites, which can be difficult to produce in other types of cells.

Steviol Glycoside Compositions

[00116] Steviol glycosides do not necessarily have equivalent performance in different food systems. It is therefore desirable to have the ability to direct the synthesis to steviol glycoside compositions of choice. Recombinant hosts described herein can produce compositions that are selectively enriched for specific steviol glycosides (e.g., RebD) and have a consistent taste profile. Thus, the recombinant hosts described herein can facilitate the production of compositions that are tailored to meet the sweetening profile desired for a given food product and that have a proportion of each steviol glycoside that is consistent from batch to batch. Hosts described herein do not produce the undesired plant by-products found in *Stevia* extracts. Thus, steviol glycoside compositions produced by the recombinant hosts described herein are distinguishable from compositions derived from *Stevia* plants.

[00117] The amount of an individual steviol glycoside (e.g., RebA, RebB, RebD, or RebM) produced can be from about 1 mg/L to about 2,800 mg/L, e.g., about 1 to about 10 mg/L, about 3 to about 10 mg/L, about 5 to about 20 mg/L, about 10 to about 50 mg/L, about 10 to about 100 mg/L, about 25 to about 500 mg/L, about 100 to about 1,500 mg/L, or about 200 to about 1,000 mg/L, at least about 1,000 mg/L, at least about 1,200 mg/L, at least about at least 1,400 mg/L, at least about 1,600 mg/L, at least about 1,800 mg/L, or at least about 2,800 mg/L. In some aspects, the amount of an individual steviol glycoside can exceed 2,800 mg/L. The amount of a combination of steviol glycosides (e.g., RebA, RebB, RebD, or RebM) produced can be from about 1 mg/L to about 6,000 mg/L, e.g., about 200 to about 1,500, at least about 2,000 mg/L, at least about 3,000 mg/L, at least about 4,000 mg/L, at least about 5,000 mg/L, or at least about 6,000 mg/L. In some aspects, the amount of a combination of steviol glycosides can exceed 6,000 mg/L. In general, longer culture times will lead to greater amounts of product. Thus, the recombinant microorganism can be cultured for from 1 day to 7 days, from 1 day to 5 days, from 3 days to 5 days, about 3 days, about 4 days, or about 5 days.

[00118] It will be appreciated that the various genes and modules discussed herein can be present in two or more recombinant microorganisms rather than a single microorganism. When a plurality of recombinant microorganisms is used, they can be grown in a mixed culture to produce steviol and/or steviol glycosides. For example, a first microorganism can comprise one or more biosynthesis genes for producing steviol and null mutations in a first group of endogenous transporters, while a second microorganism comprises steviol glycoside biosynthesis genes and null mutations in a second group of endogenous transporters. The product produced by the second, or final microorganism is then recovered. It will also be appreciated that in some embodiments, a recombinant microorganism is grown using nutrient sources other than a culture medium and utilizing a system other than a fermenter.

[00119] Alternatively, the two or more microorganisms each can be grown in a separate culture medium and the product of the first culture medium, e.g., steviol, can be introduced into second culture medium to be converted into a subsequent intermediate, or into an end product such as RebA. The product produced by the second, or final microorganism is then recovered. The microorganisms can have the same or a different group of mutations in endogenous transporters. It will also be appreciated that in some embodiments, a recombinant microorganism is grown using nutrient sources other than a culture medium and utilizing a system other than a fermenter.

[00120] Steviol glycosides do not necessarily have equivalent performance in different food systems. It is therefore desirable to have the ability to direct the synthesis to steviol glycoside compositions of choice. Recombinant hosts described herein can produce compositions that are selectively enriched for specific steviol glycosides (e.g., RebD) and have a consistent taste profile. Thus, the recombinant microorganisms described herein can facilitate the production of compositions that are tailored to meet the sweetening profile desired for a given food product and that have a proportion of each steviol glycoside that is consistent from batch to batch. Microorganisms described herein do not produce the undesired plant byproducts found in *Stevia* extracts. Thus, steviol glycoside compositions produced by the recombinant microorganisms described herein are distinguishable from compositions derived from *Stevia* plants.

[00121] Steviol glycosides and compositions obtained by the methods disclosed herein can be used to make food products, dietary supplements and sweetener compositions. See, e.g., WO 2011/153378, WO 2013/022989, WO 2014/122227, and WO 2014/122328, each of which has been incorporated by reference in its entirety.

[00122] For example, substantially pure steviol or steviol glycoside such as RebM or RebD can be included in food products such as ice cream, carbonated beverages, fruit juices, yogurts, baked goods, chewing gums, hard and soft candies, and sauces. Substantially pure steviol or steviol glycoside can also be included in non-food products such as pharmaceutical products, medicinal products, dietary supplements and nutritional supplements. Substantially pure steviol or steviol glycosides may also be included in animal feed products for both the agriculture industry and the companion animal industry. Alternatively, a mixture of steviol and/or steviol glycosides can be made by culturing recombinant microorganisms separately, each producing a specific steviol or steviol glycoside, recovering the steviol or steviol glycoside in substantially pure form from each microorganism and then combining the compounds to obtain a mixture comprising each compound in the desired proportion. The recombinant microorganisms described herein permit more precise and consistent mixtures to be obtained compared to current Stevia products. For example, recombinant microorganisms described herein can express transporters specific for transport of a particular rebaudioside into the culture medium. When a transporter is specific for a particular rebaudioside it will enrich the concentration of that compound in the fermentation broth, preventing it from being further reacted to a different compound, and by selectively transporting the rebaudioside into the fermentation broth it will make it easier to recover from the other rebaudiosides and therefore making the process more efficient.

[00123] In another alternative, a substantially pure steviol or steviol glycoside can be incorporated into a food product along with other sweeteners, e.g. saccharin, dextrose, sucrose, fructose, erythritol, aspartame, sucralose, monatin, or acesulfame potassium. The weight ratio of steviol or steviol glycoside relative to other sweeteners can be varied as desired to achieve a satisfactory taste in the final food product. See, e.g., U.S. 2007/0128311. In some embodiments, the steviol or steviol glycoside may be provided with a flavor (e.g., citrus) as a flavor modulator.

[00124] Compositions produced by a recombinant microorganism described herein can be incorporated into food products. For example, a steviol glycoside composition produced by a recombinant microorganism can be incorporated into a food product in an amount ranging from about 20 mg steviol glycoside/kg food product to about 1800 mg steviol glycoside/kg food product on a dry weight basis, depending on the type of steviol glycoside and food product. For example, a steviol glycoside composition produced by a recombinant microorganism can be incorporated into a dessert, cold confectionary (e.g., ice cream), dairy product (e.g., yogurt), or beverage (e.g., a carbonated beverage) such that the food product has a maximum of 500 mg steviol glycoside/kg food on a dry weight basis. A steviol

glycoside composition produced by a recombinant microorganism can be incorporated into a baked good (e.g., a biscuit) such that the food product has a maximum of 300 mg steviol glycoside/kg food on a dry weight basis. A steviol glycoside composition produced by a recombinant microorganism can be incorporated into a sauce (e.g., chocolate syrup) or vegetable product (e.g., pickles) such that the food product has a maximum of 1000 mg steviol glycoside/kg food on a dry weight basis. A steviol glycoside composition produced by a recombinant microorganism can be incorporated into a bread such that the food product has a maximum of 160 mg steviol glycoside/kg food on a dry weight basis. A steviol glycoside composition produced by a recombinant microorganism, plant, or plant cell can be incorporated into a hard or soft candy such that the food product has a maximum of 1600 mg steviol glycoside/kg food on a dry weight basis. A steviol glycoside composition produced by a recombinant microorganism, plant, or plant cell can be incorporated into a processed fruit product (e.g., fruit juices, fruit filling, jams, and jellies) such that the food product has a maximum of 1000 mg steviol glycoside/kg food on a dry weight basis.

[00125] For example, such a steviol glycoside composition can have from 90-99% RebA and an undetectable amount of stevia plant-derived contaminants, and be incorporated into a food product at from 25-1600 mg/kg, e.g., 100-500 mg/kg, 25-100 mg/kg, 250-1000 mg/kg, 50-500 mg/kg or 500-1000 mg/kg on a dry weight basis.

[00126] Such a steviol glycoside composition can be a RebB-enriched composition having greater than 3% RebB and be incorporated into the food product such that the amount of RebB in the product is from 25-1600 mg/kg, e.g., 100-500 mg/kg, 25-100 mg/kg, 250-1000 mg/kg, 50-500 mg/kg or 500-1000 mg/kg on a dry weight basis. Typically, the RebB-enriched composition has an undetectable amount of stevia plant-derived contaminants.

[00127] Such a steviol glycoside composition can be a RebD-enriched composition having greater than 3% RebD and be incorporated into the food product such that the amount of RebD in the product is from 25-1600 mg/kg, e.g., 100-500 mg/kg, 25-100 mg/kg, 250-1000 mg/kg, 50-500 mg/kg or 500-1000 mg/kg on a dry weight basis. Typically, the RebD-enriched composition has an undetectable amount of stevia plant-derived contaminants.

[00128] Such a steviol glycoside composition can be a RebE-enriched composition having greater than 3% RebE and be incorporated into the food product such that the amount of RebE in the product is from 25-1600 mg/kg, e.g., 100-500 mg/kg, 25-100 mg/kg, 250-1000 mg/kg, 50-500 mg/kg or 500-1000 mg/kg on a dry weight basis. Typically, the

RebE-enriched composition has an undetectable amount of stevia plant-derived contaminants.

[00129] Such a steviol glycoside composition can be a RebM-enriched composition having greater than 3% RebM and be incorporated into the food product such that the amount of RebM in the product is from 25-1600 mg/kg, *e.g.*, 100-500 mg/kg, 25-100 mg/kg, 250-1000 mg/kg, 50-500 mg/kg or 500-1000 mg/kg on a dry weight basis. Typically, the RebM-enriched composition has an undetectable amount of stevia plant-derived contaminants.

[00130] In some embodiments, a substantially pure steviol or steviol glycoside is incorporated into a tabletop sweetener or “cup-for-cup” product. Such products typically are diluted to the appropriate sweetness level with one or more bulking agents, *e.g.*, maltodextrins, known to those skilled in the art. Steviol glycoside compositions enriched for RebA, RebB, RebD, RebE, or RebM, can be package in a sachet, for example, at from 10,000 to 30,000 mg steviol glycoside/kg product on a dry weight basis, for tabletop use.

[00131] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

[00132] The Examples that follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1. LC-MS Analytical Procedures

[00133] The LC-MS methods described here are oriented towards the separation, general detection and potential identification of chemicals of particular masses (*i.e.* steviol glycosides) in the presence of a mixture (*i.e.* culture media). LC-MS analyses were performed on: (A) an UltiMate® 3000-TSQ (Thermo Fisher Scientific); (B) a 1290 Infinity – 6130SQ (Agilent); or (C) an Acquity –XevoTQD (Waters) sytem. Specific methods used for each system are described below.

[00134] Method A: LC-MS analyses were performed using an UltiMate® 3000 UPLC system (Dionex) fitted with a waters ACQUITY UPLC® BEH shield RP18 column (2.1 x 50 mm, 1.7 µm particles, 130 Å pore size) connected to a TSQ Quantum® Access (ThermoFisher Scientific) triple quadropole mass spectrometer with a heated electrospray ion (HESI) source, unless otherwise indicated. Elution was carried out using a mobile phase of eluent B (MeCN with 0.1% Formic acid) and eluent A (water with 0.1% Formic acid) by

increasing the gradient from 25% to 47 % B from min. 0.0 to 4.0, increasing 47% to 100% B in min. 4.0 to 5.0, holding 100% B from min. 5.0 to 6.5 re-equilibration. The flow rate was 0.4 mL/min and the column temperature 35°C. The steviol glycosides were detected using SIM (Single Ion Monitoring) with the following m/z-traces.

Table 1. MS analytical information for Steviol Glycosides

Description	Exact Mass	m/z trace	compound (typical t_R in min)
Steviol + 1 Glucose	[M+H] ⁺ 481.2796 [M+Na] ⁺ 503.2615	481.2± 0.5 503.1± 0.5	19-SMG (2.29), 13-SMG (3.5)
Steviol + 2 Glucose	[M+Na] ⁺ 665.3149	665± 0.5	Rubusoside (2.52) Steviol-1,2-bioside (2.92) Steviol-1,3-bioside (2.28)
Steviol + 3 Glucose	[M+Na] ⁺ 827.3677	827.4 ± 0.5	1,2-Stevioside (2.01) 1,3-Stevioside (2.39) RebB (2.88)
Steviol + 4 Glucose	[M+Na] ⁺ 989.4200	989.4 ± 0.5	RebA (2.0)
Steviol + 5 Glucose	[M+Na] ⁺ 1151.4728	1151.4 ± 0.5	RebD (1.1)
Steviol + 6 Glucose	[M+Na] ⁺ 1313.5257	1313.5 ± 0.5	RebM (1.3)

[00135] The levels of steviol glycosides were quantified by comparing with calibration curves obtained with authentic standards from LGC Standards. For example, standard solutions of 0.5 to 100 µM RebA were typically utilized to construct a calibration curve.

[00136] Method B: A second analytical method was performed on the Agilent system 1290 Infinity fitted with a waters ACQUITY UPLC® BEH shield RP18 column (2.1 x 50 mm, 1.7 µm particles, 130 Å pore size, Waters) was connected to a 6130 single quadrupol mass detector (Agilent) with a APCI ion source. Elution was carried out using a mobile phase of eluent B (MeCN with 0.1% Formic acid) and eluent A (water with 0.1% Formic acid) by increasing the gradient from 23% to 47 % B from min. 0.0 to 4.0, increasing 47% to 100% B in min. 4.0 to 5.0, holding 100% B from min. 5.0 to 6.5 re-equilibration. The flow rate was 0.6 mL/min and the column temperature 50°C. The steviol glycosides were detected using SIM (Single Ion Monitoring) with the following m/z-traces.

Table 2. MS analytical information for Steviol Glycosides

SIM trace No	time window	m/z trace	Exact Mass	Description	compound (typical t_R in min)
1	0.0-1.51min	1289.5	[M-H] ⁻	Steviol	RebM (0.91)

			1289.5281	+ 6 Glucose	
	1.51-1.90 min	687.3	[M+HCOOH-H] ⁻ 687.3217	Steviol + 2 Glucose	Rubusoside
	1.90-5.0 min	641.0	[M-H] ⁻ 641.3168	Steviol + 2 Glucose	1,2-Stevioside (1.44) 1,3-stevioside (1.74)
2	0.0-1.0 min	1127.4	[M-H] ⁻ 1127.4752	Steviol + 5 Glucose	RebD (0.81)
	1.0-5.0 min	525.3	[M-HCOOH-H] ⁻ 525.2689	Steviol + 1 Glucose	19SMG (2.49) 13SMG (2.65)
3	0.0-2.8 min	965.4	[M-H] ⁻ 965.4224	Steviol + 4 Glucose	RebA (1.42)
4	0.0-3.2 min	803.4	[M-H] ⁻ 803.3696	Steviol + 2 Glucose	1,2-Stevioside (2.16) 1,3-Stevioside (2.34) RebB (2.13)

[00137] The levels of steviol glycosides were quantified by comparing with calibration curves obtained with authentic standards from LGC Standards. For example, standard solutions of 0.3 to 25 μ M RebA were typically utilized to construct a calibration curve.

[00138] Method C: A third analytical method used was LC-MS analyses performed using a Waters ACQUITY UPLC (Waters Corporation, Milford, MA) with Waters ACQUITY UPLC® BEH C18 column (2.1 x 50 mm, 1.7 μ m particles, 130 Å pore size) coupled to a Waters ACQUITY TQD triple quadrupole mass spectrometer with electrospray ionization (ESI) in negative mode. Compound separation was achieved by a gradient of the two mobile phases A (water with 0.1% formic acid) and B (MeCN with 0.1% formic acid) by increasing from 20% to 50 % B between 0.3 to 2.0 min, increasing to 100% B at 2.01 min, holding 100% B for 0.6 min and re-equilibrate for another 0.6 min. The flow rate was 0.6 mL/min and the column temperature 55°C. RebD (m/z 1127.5), RebM (m/z 1289.5), rebaudioside A (m/z 965.4) and RebB (m/z 803.4) were monitored using SIM (Single Ion Monitoring) and quantified by comparing with authentic standards.

Example 2. Construction of a Steviol Glycoside-Producing Yeast Strain

[00139] Steviol glycoside-producing *S. cerevisiae* strains were constructed as described in WO 2011/153378, WO 2013/022989, WO 2014/122227, and WO 2014/122328, each of which is incorporated by reference in its entirety. For example, a yeast strain comprising a recombinant gene encoding a *Synechococcus* sp. GGPPS polypeptide (SEQ ID NO:1, SEQ ID NO:149), a recombinant gene encoding a truncated *Zea mays* CDPS polypeptide (SEQ ID NO:2, SEQ ID NO:150), a recombinant gene encoding an *A. thaliana* KS polypeptide (SEQ ID NO:3, SEQ ID NO:151), a recombinant gene encoding a recombinant *S. rebaudiana* KO1 polypeptide (SEQ ID NO:4, SEQ ID NO:152), a recombinant gene encoding

an *A. thaliana* ATR2 polypeptide (SEQ ID NO:5, SEQ ID NO:153), a recombinant gene encoding an *O. sativa* EUGT11 polypeptide (SEQ ID NO:12; SEQ ID NO:148), a recombinant gene encoding an SrKAHe1 polypeptide (SEQ ID NO:6, SEQ ID NO:154), a recombinant gene encoding an *S. rebaudiana* CPR8 polypeptide (SEQ ID NO:7, SEQ ID NO:155), a recombinant gene encoding an *S. rebaudiana* UGT85C2 polypeptide (SEQ ID NO:8, SEQ ID NO:156), a recombinant gene encoding an *S. rebaudiana* UGT74G1 polypeptide (SEQ ID NO:9, SEQ ID NO:157), a recombinant gene encoding an *S. rebaudiana* UGT76G1 polypeptide (SEQ ID NO:10, SEQ ID NO:158), and a recombinant gene encoding an *S. rebaudiana* UGT91D2 variant (or functional homolog), UGT91D2e-b (SEQ ID NO:11, SEQ ID NO:159) polypeptide produced steviol glycosides. As analyzed by LC-MS (Method C) following DMSO-extraction of total steviol glycosides from the whole cell and broth mixture (total production), the strain produced between 18-21 $\mu\text{g/mL}$ or 1-1.5 $\mu\text{g/mL/OD}_{600}$ RebM after growth for five days in 1 mL SC (Synthetic Complete) media at 30°C with 400 rpm shaking in deep-well plates. See Table 3.

Table 3. Steviol glycoside production in a representative *S. cerevisiae* strain comprising genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

RebB ($\mu\text{g/mL/OD}_{600}$)	RebA ($\mu\text{g/mL/OD}_{600}$)	RebD ($\mu\text{g/mL/OD}_{600}$)	RebM ($\mu\text{g/mL/OD}_{600}$)	Normalized by OD ₆₀₀
0.21	0.33	0.33	1.3	Average
0.028	0.054	0.032	0.14	Std Deviation
RebB ($\mu\text{g/mL}$)	RebA ($\mu\text{g/mL}$)	RebD ($\mu\text{g/mL}$)	RebM ($\mu\text{g/mL}$)	
3.1	4.9	5.0	19.0	Average
0.42	0.81	0.48	2.1	Std Deviation

[00140] A second strain, which comprised additional copies of the genes of the first strain, was analyzed for steviol glycoside production. The second strain produced RebD and RebM as primary steviol glycosides, although at higher levels than the first strain.

[00141] As analyzed by LC-MS (Method C) following DMSO-extraction of total steviol glycosides from the whole cell and broth mixture (total production), the second strain produced between 60-80 $\mu\text{g/mL}$ or 4-6 $\mu\text{g/mL/OD}_{600}$ RebM, after growth for five days in 1 mL SC media at 30°C with 400 rpm shaking in deep-well plates. Production of RebA, RebB, RebD and RebM by the second strain is shown in Table 4.

Table 4. Steviol glycoside production in an *S. cerevisiae* strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

RebA ($\mu\text{g/mL/OD}_{600}$)	RebB ($\mu\text{g/mL/OD}_{600}$)	RebD ($\mu\text{g/mL/OD}_{600}$)	RebM ($\mu\text{g/mL/OD}_{600}$)	Normalized by OD_{600}
2.1	0.67	1.6	4.8	Average
0.66	0.21	0.75	2.3	Std Deviation
RebA ($\mu\text{g/mL}$)	RebB ($\mu\text{g/mL}$)	RebD ($\mu\text{g/mL}$)	RebM ($\mu\text{g/mL}$)	
31.0	10.1	23.7	72.5	Average
9.9	3.1	11.3	34.4	Std Deviation

Example 3. Knockout of Yeast Endogenous Transport Genes and Transport-Related Genes

[00142] Observations from deep-well studies of Example 2 and similar strains indicated that the fraction of RebA, RebB, RebD or RebM in the supernatant changes with time, and the effect was determined not to be the result of cell lysis. To determine the effect of various transporters on steviol glycoside excretion in *S. cerevisiae*, deletion cassettes for homologous recombination were obtained by designing primers annealing approximately 200 bp upstream and downstream of the open reading frame (ORF) and then amplifying the ORF-specific deletion cassette from the *S. cerevisiae* deletion collection. The candidate genes selected include identified ORFs with relation to transport or comprising membrane spanning domains, regardless of subcellular localization. In the resulting colonies, the presence of the deletion cassette at the correct locus was verified by colony PCR. A maximum of 6 clones of each deletion was frozen down as freezer stock. All samples for analysis were initiated from the freezer stock and grown in SC medium for 5 days (30°C, shaking 400 rpm) prior to harvest and extraction of samples for LC-MS. Samples were analyzed for the presence of RebA, RebB, RebD and RebM in the culture broth lacking cells (Supernatant) as well as in the whole cell and broth mixture (Total production).

[00143] Concentrations of total and supernatant RebA, RebB, RebD and RebM were compared to the levels in a control steviol glycoside-producing strain. The amounts of RebA, RebB, RebD and RebM in each sample were normalized to the control strain by dividing the value of a particular steviol glycoside with the corresponding value for the control strain, thereby calculating a percentage to the control strain, where 1 equals 100 percent. The “ideal candidate” would exhibit a decrease in RebA, RebB, RebD and/or RebM levels in the supernatant, as compared to the control steviol glycoside-producing strain, without decreasing RebA, RebB, RebD, and/or RebM total production.

[00144] The effect of yeast gene knockouts on transport of higher molecular weight steviol glycosides into the culture medium was tested in a strain that produces steviol glycosides, such as the strains described in Example 2. Disruption of each specific transporter gene was performed by homologous recombination. After 5 days of growth in 1 mL SC medium at 30°C and 400 rpm, cells were harvested. A 50 µL aliquot of the culture was mixed with an equal volume of 100% DMSO, vortexed, and heated to 80°C for 10 min. The suspension was then centrifuged to remove cell debris. 60 µL of the mixture were analyzed by LC-MS as the “Total” sample. The remaining culture was then centrifuged to pellet cells. An aliquot of 50 µL was removed from the supernatant (*i.e.*, the culture medium) and mixed with an equal volume of 100% DMSO. The suspension was heated to 80°C for 10 min and centrifuged. 60 µL of the the mixture were analysed by LC-MS as the “Supernatant” sample. The amounts of higher molecular weight steviol glycosides (including RebA, RebB, RebD, RebM) were measured by LC-MS (Method C), as described in Example 1.

[00145] The data demonstrate that disruption of a single endogenous yeast transporter gene in a steviol glycoside-producing strain resulted in a decrease in the level of various steviol glycosides in the supernatant of the culture media, as evaluated by the normalized amount transported into the supernatant (see Tables 5-10). Tables 5-10 comprise lists of transport related genes that were knocked out in a steviol glycoside-producing strain. More specifically, Table 5 comprises a compiled list of genes by ordered locus name found to affect steviol glycoside excretion in steviol glycoside-producing strains and are therefore identified as having a role in steviol glycoside excretion. When the specified genes were knocked out, a more than 40% decrease in either the supernatant alone or in the ratio of supernatant/total production of RebA, RebB, RebD, and/or RebM was observed. This corresponded approximately to more than 2 standard deviations removed from the mean of a control steviol glycoside-producing strain (a value of 1 equals 100 percent of the control strain, whereas a value of 0.5 indicates a 50% decrease).

[00146] Table 6 comprises a compiled list of genes by ordered locus name found to affect steviol glycoside excretion in steviol glycoside-producing strains and are therefore identified as having a role in steviol glycoside excretion. When knocked out, these genes caused a mean of between 20-40% decrease in either the supernatant alone or in the ratio of supernatant/total production. This corresponded to approximately between 1 and 2 standard deviations removed from the mean of the control strain (a value of 1 equals 100 percent of the control strain, whereas a value of 0.5 indicates a 50% decrease).

Table 5A. Transport related genes with over a 40% decrease in Reb A, RebB, RebD or RebM levels compared to a control steviol glycoside-producing strain.

SEQ ID No.	Ordered Locus Name	Family	Description	Gene name	Uniprot Accession No.
13	YBR180W	MFS	Secondary Transporter	DTR1	P38125
14	YAL067C	MFS	Secondary Transporter	SEO1	P39709
15	YBL089W	AAAP	Secondary Transporter	AVT5	P38176
16	YBL099W	F-ATPase	ATP-Dependent	ATP1	P07251
17	YBR241C	MFS	Secondary Transporter		P38142
18	YBR294W	SulP	Secondary Transporter	SUL1	P38359
19	YCL069W	MFS	Secondary Transporter	VBA3	P25594
20	YCR028C	MFS	Secondary Transporter	FEN2	P25621
21	YCR075C	LCT	Secondary Transporter	ERS1	P17261
22	YDL128W	CaCA	Secondary Transporter	VCX1	Q99385
23	YDL185W	F-ATPase	ATP-Dependent	VMA1	P17255
24	YDL194W	MFS	Secondary Transporter	SNF3	P10870
25	YDL210W	APC	Secondary Transporter	UGA4	P32837
26	YDR061W	ABC	ATP-Dependent		Q12298
27	YDR093W	P-ATPase	ATP-Dependent	DNF2	Q12675
28	YDR338C	MOP/MATE	Secondary Transporter		Q05497
29	YDR406W	ABC	ATP-Dependent	PDR15	Q04182
30	YDR536W	MFS	Secondary Transporter	STL1	P39932
31	YEL031W	P-ATPase	ATP-Dependent	SPF1	P39986
32	YER166W	P-ATPase	ATP-Dependent	DNF1	P32660
33	YFL011W	MFS	Secondary Transporter	HXT10	P43581
34	YGL006W	P-ATPase	ATP-Dependent	PMC1	P38929
35	YGL013C	Transcription factor		PDR1	P12383
36	YGL255W	ZIP	Secondary Transporter	ZRT1	P32804
37	YGR125W	SulP	Secondary Transporter		P53273
38	YGR181W	MPT	ATP-Dependent	TIM13	P53299
39	YGR217W	VIC	Ion Channels	CCH1	P50077
40	YGR224W	MFS	Secondary Transporter	AZR1	P50080
41	YGR281W	ABC	ATP-Dependent	YOR1	P53049
42	YHL016C	SSS	Secondary Transporter	DUR3	P33413
43	YIL088C	AAAP	Secondary Transporter	AVT7	P40501
44	YJL093C	VIC	Ion Channels	TOK1	P40310
45	YJL094C	CPA2	Secondary Transporter	KHA1	P40309
46	YJL108C	ThrE	Secondary Transporter	PRM10	P42946
47	YJL212C	OPT	Secondary Transporter	OPT1	P40897
48	YJR106W	CaCA	Secondary Transporter	ECM27	P47144

Table 5B. Continued list of Transport related genes with over a 40% decrease in Reb A, RebB, RebD or RebM levels compared to a control steviol glycoside-producing strain.

No.	Ordered Locus Name	Family	Description	Gene name	Uniprot Accession No.
49	YJR160C	MFS	Secondary Transporter	MPH3	P0CE00
50	YKL064W	MIT	Ion Channels	MNR2	P35724
51	YKR050W	Trk	Secondary Transporter	TRK2	P28584
52	YKR105C	MFS	Secondary Transporter	VBA5	P36172
53	YKR106W	MFS	Secondary Transporter	GEX2	P36173
54	YLR447C	F-ATPase	ATP-Dependent	VMA6	P32366
55	YML116W	MFS	Secondary Transporter	SNQ1/ATR1	P13090
56	YMR034C	BASS	Secondary Transporter		Q05131
57	YMR056C	MC	Secondary Transporter	AAC1	P04710
58	YMR253C	DMT	Secondary Transporter		Q04835
59	YNL065W	MFS	Secondary Transporter	AQR1	P53943
60	YNL070W	MPT	ATP-Dependent	TOM7	P53507
61	YNL083W	MC	Secondary Transporter	SAL1	D6W196
62	YNL095C	AEC	Secondary Transporter		P53932
63	YNL121C	MPT	ATP-Dependent	TOM70	P07213
64	YNL142W	Amt	Ion Channels	MEP2	P41948
65	YOL020W	APC	Secondary Transporter	TAT2	P38967
66	YOL075C	ABC	ATP-Dependent		Q08234
67	YOL077W-A	F-ATPase	ATP-Dependent	ATP19	P81451
68	YOL122C	Nramp	Secondary Transporter	SMF1	P38925
69	YOR079C	ZIP	Secondary Transporter	ATX2	Q12067
70	YOR087W	TRP-CC	Ion Channels	YVC1	Q12324
71	YOR092W	AEC	Secondary Transporter	ECM3	Q99252
72	YOR130C	MC	Secondary Transporter	ORT1	Q12375
73	YOR222W	MC	Secondary Transporter	ODC2	Q99297
74	YOR291W	P-ATPase	ATP-Dependent	YPK9	Q12697
75	YOR306C	MFS	Secondary Transporter	MCH5	Q08777
76	YOR316C	CDF	Secondary Transporter	COT1	P32798
77	YOR334W	MIT	Ion Channels	MRS2	Q01926
78	YPL078C	F-ATPase	ATP-Dependent	ATP4	P05626
79	YPL270W	ABC	ATP-Dependent	MDL2	P33311
80	YPL274W	APC	Secondary Transporter	SAM3	Q08986
81	YPR003C	SuIP	Secondary Transporter		P53394
82	YPR011C	MC	Secondary Transporter		Q12251
83	YPR058W	MC	Secondary Transporter	YMC1	P32331
84	YPR128C	MC	Secondary Transporter	ANT1	Q06497
85	YPR201W	ACR3	Secondary Transporter	ARR3	Q06598

Table 6A. Transport related genes with a 20-40% decrease in Reb A, RebB, RebD or RebM levels compared to a control steviol glycoside-producing strain.

SEQ ID No.	Ordered Locus Name	Family	Description	Gene name	Uniprot Accession No.
86	YBR008C	MFS	Secondary Transporter	FLR1	P38124
87	YBR021W	NCS1	Secondary Transporter	FUR4	P05316
88	YBR043C	MFS	Secondary Transporter	QDR3	P38227
89	YBR287W	AEC	Secondary Transporter		P38355
90	YBR295W	P-ATPase	ATP-Dependent	PCA1	P38360
91	YBR296C	PiT	Secondary Transporter	PHO89	P38361
92	YCL038C	MFS	Secondary Transporter	ATG22	P25568
93	YCR011C	ABC	ATP-Dependent	ADP1	P25371
94	YDL054C	MFS	Secondary Transporter	MCH1	Q07376
95	YDL100C	ArsAB	ATP-Dependent	GET3	Q12154
96	YDL245C	MFS	Secondary Transporter	HXT15	P54854
97	YDL247W	MFS	Secondary Transporter	MPH2	P0CD99
98	YDR011W	ABC	ATP-Dependent	SNQ2	P32568
99	YDR292C	IISP	ATP-Dependent	SRP101	P32916
100	YDR497C	MFS	Secondary Transporter	ITR1	P30605
101	YEL006W	MC	Secondary Transporter	YEA6	P39953
102	YEL027W	F-ATPase	ATP-Dependent	VMA3	P25515
103	YEL065W	MFS	Secondary Transporter	SIT1	P39980
104	YER019C-A	IISP	ATP-Dependent	SBH2	P52871
105	YER053C	MC	Secondary Transporter	PIC2	P40035
106	YER119C	AAAP	Secondary Transporter	AVT6	P40074
107	YFL028C	ABC	ATP-Dependent	CAF16	P43569
108	YFR045W	MC	Secondary Transporter		P43617
109	YGL084C	GUP	Secondary Transporter	GUP1	P53154
110	YGL104C	MFS	Secondary Transporter	VPS73	P53142
111	YGL114W	OPT	Secondary Transporter		P53134
112	YGL167C	P-ATPase	ATP-Dependent	PMR1	P13586
113	YGR257C	MC	Secondary Transporter	MTM1	P53320
114	YHL035C	ABC	ATP-Dependent	VMR1	P38735
115	YHL036W	APC	Secondary Transporter	MUP3	P38734

Table 6B. Continued list of Transport related genes with a 20-40% decrease in Reb A, RebB, RebD or RebM levels compared to a control steviol glycoside-producing strain.

No.	Ordered Locus Name	Family	Description	Gene name	Accession No.
116	YHR002W	MC	Secondary Transporter	LEU5	P38702
117	YHR096C	MFS	Secondary Transporter	HXT5	P38695
118	YIL006W	MC	Secondary Transporter	YIA6	P40556
119	YIL120W	MFS	Secondary Transporter	QDR1	P40475
120	YIL121W	MFS	Secondary Transporter	QDR2	P40474
121	YIL166C	MFS	Secondary Transporter	SOA1	P40445
122	YJL133W	MC	Secondary Transporter	MRS3	P10566
123	YJL219W	MFS	Secondary Transporter	HXT9	P40885
124	YKL016C	F-ATPase	ATP-Dependent	ATP7	P30902
125	YKL050C	MIT	Ion Channels		P35736
126	YKL120W	MC	Secondary Transporter	OAC1	P32332
127	YKL146W	AAAP	Secondary Transporter	AVT3	P36062
128	YKL209C	ABC	ATP-Dependent	STE6	P12866
129	YKR039W	APC	Secondary Transporter	GAP1	P19145
130	YLR411W	Ctr	Ion Channels	CTR3	Q06686
131	YML038C	DMT	Secondary Transporter	YMD8	Q03697
132	YMR166C	MC	Secondary Transporter		Q03829
133	YMR279C	MFS	Secondary Transporter		Q03263
134	YNL003C	MC	Secondary Transporter	PET8	P38921
135	YNL268W	APC	Secondary Transporter	LYP1	P32487
136	YNR055C	MFS	Secondary Transporter	HOL1	P53389
137	YOL158C	MFS	Secondary Transporter	ENB1	Q08299
138	YOR100C	MC	Secondary Transporter	CRC1	Q12289
139	YOR153W	ABC	ATP-Dependent	PDR5	P33302
140	YOR271C	MTC	Secondary Transporter	FSF1	Q12029
141	YOR273C	MFS	Secondary Transporter	TPO4	Q12256
142	YOR307C	DMT	Secondary Transporter	SLY41	P22215
143	YOR332W	F-ATPase	ATP-Dependent	VMA4	P22203
144	YOR348C	APC	Secondary Transporter	PUT4	P15380
145	YPL036W	P-ATPase	ATP-Dependent	PMA2	P19657

[00147] Steviol glycoside exporter candidates were selected from the data based on two selection criteria for each steviol glycoside measured (*i.e.*, two methods of normalizing expression).

[00148] Transporter selection criterion 1 corresponded to selection based on the level of high molecular weight steviol glycosides (RebA, RebB, RebD, or RebM) available in the supernatant, as well as the total production of the said steviol glycoside. Both values were normalized to the value of the corresponding steviol glycoside-producing control strain. The control level was set to 1, and the corresponding steviol glycoside level was calculated as a

percentage of the control. For Ordered Locus Names (*i.e.*, genes) of interest, the steviol glycoside available in the supernatant should be below 0.6 (below 60% of the control) or between 0.8-0.6 (80-60% of the control). To avoid false positives or a bias towards transporters that decrease the production in general, the calculation had an additional requirement that the total production had to be similar to the control. In the current calculation, production was set to be between 0.85 and 1.15 of the control, when the control is set to 1. In this regard, steviol glycoside production levels did not affect results. Table 7 shows the supernatant/total ratio for each candidate that fulfills the selection criteria.

[00149] Transporter selection criterion 2 corresponded to selection based on the ratio of high molecular weight steviol glycosides (RebA, RebB, RebD, or RebM) in the supernatant relative to total production of the said steviol glycoside. The supernatant-to-total production ratio was normalized to the ratio of the corresponding steviol glycoside-producing strain control. The control level was set to 1, and the corresponding steviol glycoside ratio was calculated as a percentage of the control. For Ordered Locus Names (*i.e.*, genes) of interest, the supernatant-to-total production ratio for a given steviol glycoside should be below 0.6 (below 60% of the control) or between 0.8-0.6 (80-60% of the control). To avoid false positives or a bias towards transporters that decrease the production in general, the calculation had an additional requirement that the total production had to be similar to the control. In the current calculation, production was set to be between 0.85 and 1.15 of the control, when the control is set to 1. In this regard, steviol glycoside production levels did not affect results. Table 8 shows the supernatant/total ratio for each candidate that fulfills the selection criteria.

[00150] The data demonstrate that disruption of a single endogenous yeast transporter gene in a steviol glycoside-producing strain resulted in a decrease in the level of various steviol glycosides in the supernatant of the culture media, as evaluated by the normalized amount transported into the supernatant (see Tables 5-10), and are therefore identified as having a role in steviol glycoside excretion.

[00151] For example, deletion in a steviol glycoside-producing strain of YDL128W (SEQ ID NO:22), YDL194W (SEQ ID NO:24), YDL210W (SEQ ID NO:25), YFL011W (SEQ ID NO:33), YGL006W (SEQ ID NO:34), YGL013C (SEQ ID NO:35), YGL255W (SEQ ID NO:36), YGR181W (SEQ ID NO:38), YGR217W (SEQ ID NO:39), YIL088C (SEQ ID NO:43), YJL094C (SEQ ID NO:45), YJR106W (SEQ ID NO:48), YNL065W (SEQ ID NO:59), YNL083W (SEQ ID NO:61), YNL121C (SEQ ID NO:63), YNL142W (SEQ ID NO:64), YOR306C (SEQ ID NO:75), or YPR011C (SEQ ID NO:82) led to a measurable decrease of RebD excreted into the culture medium, indicating that each plays a role in RebD excretion.

This was confirmed by transporter selection criteria 1 and 2 (see Tables 7 and 8, RebD column).

[00152] Furthermore, for example, deletion in a steviol glycoside-producing strain of YBR180W (SEQ ID NO:13), YBR241C (SEQ ID NO:17), YCL069W (SEQ ID NO:19), YCR075C (SEQ ID NO:21), YDL128W (SEQ ID NO:22), YDL194W (SEQ ID NO:24), YDR093W (SEQ ID NO:27), YDR338C (SEQ ID NO:28), YER166W (SEQ ID NO:32), YFL011W (SEQ ID NO:33), YGL006W (SEQ ID NO:34), YGL013C (SEQ ID NO:35), YGL255W (SEQ ID NO:36), YGR217W (SEQ ID NO:39), YJL094C (SEQ ID NO:45), YJR106W (SEQ ID NO:48), YJR160C (SEQ ID NO:49), YKR106W (SEQ ID NO:53), YML116W (SEQ ID NO:55), YMR056C (SEQ ID NO:57), YNL070W (SEQ ID NO:60), YNL083W (SEQ ID NO:61), YNL095C (SEQ ID NO:62), YNL121C (SEQ ID NO:63), YOR087W (SEQ ID NO:70), YOR291W (SEQ ID NO:74), YOR306C (SEQ ID NO:75), YPL274W (SEQ ID NO:80), or YPR011C (SEQ ID NO:82) led to a measurable decrease of RebM, indicating that each plays a role in RebM excretion. This was confirmed by transporter selection criteria 1 and 2 (see Tables 7 and 8, RebM column).

[00153] Table 7 represents the calculated ratio, normalized to a steviol glycoside-producing strain comprising genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides, of supernatant/total production for each gene (by ordered locus name) deleted in the steviol glycoside-producing strain. The supernatant or supernatant/total ratio of less than 0.6 represented a more than 40% decrease in either the supernatant alone or in the ratio of supernatant/total production of RebA, RebB, RebD, or RebM, which corresponded approximately to more than 2 standard deviations removed from the mean of the control steviol glycoside-producing strain and indicates the gene as having a role in steviol glycoside transportation (Table 7). The supernatant or ratio supernatant/total of between 0.6 and 0.8 represents a 40-20% decrease in either the supernatant alone or in the ratio of supernatant/total production of RebA, RebB, RebD, or RebM, which corresponds to approximately between 1 and 2 standard deviations removed from the mean of the control steviol glycoside-producing strain, and indicates the gene as having a role in steviol glycoside transportation and/or production (Table 8). Total production of each steviol glycoside was between 0.85 and 1.15 compared to the steviol glycoside-producing strain. Table 8 shows the supernatant/total ratio for each candidate that fulfills the selection criteria.

Table 7. Transport related genes with over a 40% decrease in RebA, RebB, RebD or RebM compared to a control steviol glycoside-producing strain comprising genes

encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

	Transporter selection criterion 1 Total vs. Supernatant				Transporter selection criterion 2 Ratio Sup/Total vs. Total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YBR180W				0.486				0.486
YBR241C				0.529				0.529
YCL069W				0.519				0.519
YCR075C				0.448				0.448
YDL128W			0.459	0.405			0.459	0.405
YDL194W			0.652	0.482				0.482
YDL210W			0.000				0.000	
YDR093W				0.569				0.569
YDR338C				0.451				0.451
YEL031W		0.488				0.488		
YER166W				0.495				0.495
YFL011W			0.581	0.547			0.581	0.547
YGL006W							0.410	0.424
YGL013C			0.673	0.507				0.507
YGL255W			0.669	0.632				
YGR181W			0.419				0.419	
YGR217W			0.598	0.429			0.598	0.429
YIL088C			0.135				0.135	
YJL094C			0.568	0.525			0.568	0.525
YJR106W			0.470	0.432			0.470	0.432
YJR160C				0.689				
YKL064W		0.337				0.337		
YKR106W				0.509				0.509
YML116W				0.706				
YMR056C								0.591
YNL065W							0.571	
YNL070W				0.633				
YNL083W				0.481			0.592	0.481
YNL095C				0.610				
YNL121C			0.620	0.456				0.456
YNL142W	0.561		0.369		0.561		0.369	
YOR087W				0.611				
YOR291W				0.681				
YOR306C			0.596	0.559			0.596	0.559
YOR334W		0.520				0.520		
YPL078C		0.590				0.590		
YPL270W		0.665						
YPL274W				0.561				0.561
YPR011C			0.542	0.611			0.542	

Table 8. Transport related genes with a 20-40% decrease in Reb A, RebB, RebD or RebM compared to a control steviol glycoside-producing strain comprising genes

encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

	Transports cal 1; total vs sup				Transports cal 2; ratio sup/total vs total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YBL089W				0.739				0.739
YBR008C	0.784			0.640	0.784			0.640
YBR021W		0.731				0.731		
YBR043C	0.755			0.796	0.755			0.796
YBR180W			0.747				0.747	
YBR241C			0.688		0.798		0.688	
YBR287W	0.781	0.823	0.768		0.781		0.768	
YBR295W			0.885	0.876				
YBR296C		0.724	0.799	0.790		0.724	0.799	0.790
YCL038C		0.709		0.752		0.709		0.752
YCL069W			0.785				0.785	
YCR075C			0.634				0.634	
YDL054C			0.920					
YDL100C			0.867					
YDL194W							0.652	
YDL210W				0.834				
YDL245C	0.852							
YDL247W				0.682				0.682
YDR011W			0.852					
YDR093W	0.792	0.775	0.704		0.792	0.775	0.704	
YDR338C	0.711	0.695	0.680		0.711	0.695	0.680	
YDR497C				0.694				0.694
YEL006W				0.657			0.774	0.657
YEL065W			0.635				0.635	
YER119C				0.872				
YER166W	0.771	0.843	0.687		0.771		0.687	
YFL011W		0.787				0.787		
YFL028C			0.641				0.641	
YFR045W			0.779				0.779	
YGL006W			0.410	0.424				
YGL013C							0.673	
YGL084C		0.804						
YGL104C	0.628	0.731		0.683	0.628	0.731		0.683
YGL114W						0.796		
YGL167C	0.829							
YGL255W							0.669	0.632
YGR217W		0.801						
YGR257C	0.842							
YHL035C			0.900	0.792				0.792

	Transports cal 1; total vs sup				Transports cal 2; ratio sup/total vs total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YHL036W				0.798				0.798
YHR096C			0.879	0.798				0.798
YIL006W	0.763			0.689	0.763		0.791	0.689
YIL120W				0.814				
YIL121W			0.903					
YIL166C			0.844					
YJL212C			0.817	0.682				0.682
YJR106W	0.719				0.719			
YJR160C		0.781	0.985			0.781		0.689
YKL050C				0.896				
YKL120W				0.706				0.706
YKL146W		0.890						
YKR039W	0.763				0.763			
YKR106W		0.785	0.738			0.785	0.738	
YLR411W	0.852		0.782				0.782	
YML038C			0.724				0.724	
YML116W			0.898					0.706
YMR056C			0.675	0.591		0.786	0.675	
YMR279C				0.885				
YNL065W	0.710	0.792	0.571		0.710	0.792		
YNL070W	0.893		0.892					0.633
YNL083W			0.592					
YNL095C			0.726				0.726	0.610
YNL121C							0.620	
YNL268W		0.920						
YNR055C			0.643				0.643	
YOL122C				0.935				
YOL158C			0.848	0.728				0.728
YOR087W								0.611
YOR100C		0.916						
YOR271C		0.889	0.758	0.608			0.758	0.608
YOR273C	0.726	0.916	0.635		0.726		0.635	
YOR291W								0.681
YOR307C								0.765
YOR348C				0.644				0.644
YPL036W	0.763		0.698		0.763		0.698	
YPL078C			0.798				0.798	
YPL270W			0.746			0.665	0.746	
YPL274W	0.817	0.807	0.721				0.721	
YPR011C	0.763				0.763			0.611

[00154] The effect of yeast gene knockouts on transport of higher molecular weight steviol glycosides into the culture medium (*i.e.*, supernatant) also was tested in a steviol glycoside-producing strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides, which was described in Example 2. The data demonstrated that disruption of a single endogenous yeast transporter gene in the steviol glycoside-producing strain resulted in a decrease in the level of various steviol glycosides in the supernatant of the culture media, as evaluated by the normalized amount transported or by the supernatant-to-total-production ratio (see Tables 9 and 10, RebD column). For example, deletion in the steviol glycoside-producing strain of YDR536W (SEQ ID NO:30), YHL016C (SEQ ID NO:42), YKR050W (SEQ ID NO:51), YOR291W (SEQ ID NO:74), YOR334W (SEQ ID NO:77), YPL270W (SEQ ID NO:79), YPR058W (SEQ ID NO:83), or YPR128C (SEQ ID NO:84) led to a measurable decrease of RebD transported into the supernatant, indicating that they play a role in RebD excretion. This was confirmed by transporter selection criteria 1 and 2 (see Tables 9 and 10, RebD column).

[00155] Furthermore, for example, deletion of YAL067C (SEQ ID NO:14), YDR406W (SEQ ID NO:29), YHL016C (SEQ ID NO:42), YJL212C (SEQ ID NO:47), YKR050W (SEQ ID NO:51), YMR034C (SEQ ID NO:56), YMR253C (SEQ ID NO:58), YOL075C (SEQ ID NO:66), YOL122C (SEQ ID NO:68), YOR222W (SEQ ID NO:73), YPR003C (SEQ ID NO:81), or YPR201W (SEQ ID NO:85) led to a measurable decrease of RebM transported into the supernatant, indicating that they play a role in RebM excretion. This was confirmed by transporter selection criteria 1 and 2 (see Tables 9 and 10, RebM column).

[00156] Table 9 represents the calculated ratio, normalized to a steviol glycoside-producing strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides, of supernatant/total production for each gene (by ordered locus name) deleted in the steviol glycoside-producing strain. The supernatant or ratio supernatant/total of less than 0.6 represents a more than 40% decrease in either the supernatant alone or in the ratio of supernatant/total production of RebA, RebB, RebD, or RebM, which corresponds approximately to more than 2 standard deviations removed from the mean of a control steviol glycoside-producing strain, and indicates the gene as having a role in steviol glycoside transportation and/or production (Table 9). The supernatant or ratio supernatant/total of between 0.6 and 0.8 represents a 40-20% decrease in either the supernatant alone or in the ratio of supernatant/total production of RebA, RebB, RebD, or RebM, which corresponds to approximately between 1 and 2 standard deviations removed from the mean of the control strain, and indicates the gene as having a role in steviol

glycoside transportation and/or production, and indicates the gene as having a role in steviol glycoside transportation and/or production (Table 10). Total production of each steviol glycoside was between 0.85 and 1.15 compared to the control steviol glycoside-producing strain. Table 10 shows the supernatant/total ratio for each candidate that fulfills the selection criteria.

Table 9. Transport related genes with over a 40% decrease in Reb A, RebB, RebD or RebM compared to a control steviol glycoside-producing strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

	Transporter selection criterion 1 total vs sup				Transporter selection criterion 2 ratio sup/total vs total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YAL067C				0.541				0.541
YBL089W	0.433	0.416			0.433	0.416		
YBL099W	0.523				0.523			
YBR294W	0.495				0.495			
YCR028C		0.419				0.419		
YDL185W	0.551				0.551			
YDL210W	0.626	0.469				0.469		
YDR061W	0.482		0.471		0.482		0.471	
YDR406W				0.288				0.288
YDR536W	0.715		0.365				0.365	
YFL011W		0.444				0.444		
YGR125W		0.400				0.400		
YGR224W		0.361				0.361		
YGR281W		0.596				0.596		
YHL016C			0.427	0.296			0.427	0.296
YJL093C		0.449				0.449		
YJL108C	0.589				0.589			
YJL212C	0.442			0.461	0.442			0.461
YKR050W	0.554		0.378	0.304	0.554		0.378	0.304
YLR447C	0.512				0.512			
YMR034C	0.331			0.316	0.331			0.316
YMR253C	0.389			0.375	0.389			0.375
YOL020W	0.371				0.371			
YOL075C	0.494			0.471	0.494			0.471
YOL077W-A	0.531				0.531			
YOL122C				0.457				0.457
YOR079C	0.552				0.552			
YOR092W	0.407				0.407			
YOR130C	0.588				0.588			
YOR222W	0.469			0.457	0.469			0.457
YOR291W			0.428				0.428	
YOR334W			0.327				0.327	

	Transporter selection criterion 1 total vs sup				Transporter selection criterion 2 ratio sup/total vs total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YPL270W			0.375				0.375	
YPR003C	0.400			0.418	0.400			0.418
YPR058W			0.461				0.461	
YPR128C			0.342				0.342	
YPR201W	0.376			0.353	0.376			0.353

Table 10. Transport related genes with a 20-40% decrease in Reb A, RebB, RebD or RebM compared to a control steviol glycoside-producing strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides.

	Transports cal 1; total vs sup				Transports cal 2; ratio sup/total vs total			
	RebA	RebB	RebD	RebM	RebA	RebB	RebD	RebM
YCR011C				0.654				0.654
YDL210W			0.729		0.626		0.729	
YDR292C	0.724				0.724			
YDR536W					0.715			
YEL027W		0.799				0.799		
YER019C-A	0.789				0.789			
YER053C	0.651				0.651			
YGR256W	0.744				0.744			
YHR002W	0.795				0.795			
YJL133W	0.691				0.691			
YJL219W	0.674				0.674			
YKL016C	0.627				0.627			
YKL209C	0.721				0.721			
YKR105C					0.646			
YMR166C		0.924						
YNL003C		0.814						
YOR153W	0.801							
YOR316C					0.640			
YOR332W	0.700				0.700			

[00157] Knockouts of YDL210W (SEQ ID NO:25) and YPL270W (SEQ ID NO:79) resulted in decreased RebD excretion in the steviol glycoside-producing strain comprising genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides and the steviol glycoside-producing strain comprising additional copies of genes encoding GGPPS, truncated CDPS, KS, KO, ATR2, EUGT11, SrKAHe1, CPR8, UGT85C2, UGT74G1, UGT76G1, and EUGT11 polypeptides. As well, knockouts of YJL212C (SEQ ID NO:47) and YOL122C (SEQ ID NO:68) resulted in decreased RebM transport in both strains.

Example 4. Confirmation of Knockout of Yeast Endogenous Transport Genes by overexpression in a RebD/M-producing strain

[00158] Overexpression of a subset of the initial candidate transporters from Example 3 was performed using both plasmid-based expression and an integration cassette. First, deep-well microtiter plate culture experiments were carried out. Two transport genes were overexpressed using a plasmid in a RebD/M-producing strain in order to confirm the results from the knockout experiments. YGR181W (SEQ ID NO:38), a TIM complex, helper protein for insertion of mitochondrial inner membrane proteins, and YDR061W (SEQ ID NO:26) an ABC-like transporter were overexpressed. The data shown in Figure 2 demonstrate that the phenotype based on the knockout studies was confirmed with a plasmid based overexpression phenotype for YGR181W (SEQ ID NO:38) and YDR061W (SEQ ID NO:26) in deep-well plates.

[00159] Next, confirmation of the phenotype in fermenters was performed in additional steviol glycoside-producing strains, which were characterized by integration of YGR181W (SEQ ID NO:38) or YDR061W (SEQ ID NO:26) on chromosome XII. The steviol glycoside-producing strains were grown on defined media at 30°C in a fed-batch fermentation for about 5 days under glucose-limited conditions, and the levels of RebA, RebB, RebD, and RebM were measured using LC-MS (Method B, Example 1). The graphs shown in Figure 3 illustrate an approximate 2-fold increase in RebD and RebM transported in the culture medium for the new integration constructs, and little change in RebA and RebB transport. Overexpression of YGR181W (SEQ ID NO:38) or YDR061W (SEQ ID NO:26) resulted in improved (~2-fold) RebD and RebM transport into the culture medium (~400-500 mg/L of supernatant RebD and RebM in YGR181W (SEQ ID NO:38) and YDR061W (SEQ ID NO:26) overexpression strains versus ~250 mg/L of supernatant RebD and RebM in a control steviol glycoside-producing strain). See Figure 3A. The ratio of transported RebD as compared to the total RebD increased from 0.158 in the control strain to 0.21-0.25 with the candidate genes overexpressed. RebM transport into the culture medium was also simultaneously improved. See Figure 3.

Example 5. Overexpression of Selected Yeast Endogenous Transport Genes

[00160] Overexpression in a steviol glycoside-producing strain (as described in Example 2) using a plasmid with a constitutive promoter of the transporter genes shown in Table 11 resulted in greater than a 20% increase in excretion of RebA, RebB, RebD, and/or RebM. Results were analyzed using criterion 2 described in Example 3. Additionally, overexpression of the transporter genes shown in Table 12 resulted in greater than a 40% improvement in production of RebA, RebB, RebD, and/or RebM. Table 11 shows the supernatant/total ratio for each candidate that fulfills the selection criteria.

Table 11. Transport related genes with over a 20% increase in RebA, RebB, RebD or RebM excretion, compared to a control steviol glycoside-producing strain.

	Ratio Supernatant/Total			
	RebB	RebA	RebD	RebM
YOR079C			1.21	
YMR166C		1.36	1.53	1.38
YEL027W		1.62	1.82	1.52
YDL054C		1.45	1.38	1.31
YKL120W		1.83	1.89	1.93
YDR536W		1.79	1.80	1.76
YBL099W				1.22
YML116W		1.32	1.31	1.42
YIL166C			1.27	1.22
YKR039W			1.26	1.41
YOR307C				1.23
YKL146W		1.36	1.47	1.66
YGL167C				1.33
YJL093C				1.29
YOR306C	1.67			
YDL128W	1.85		1.29	
YOR153W	1.42		1.21	
YKL050C	1.59	1.22		
YJL094C	1.71	1.24	1.24	
YCL069W	1.59			
YOL158C	1.52			
YFL011W	1.44			

	Ratio Supernatant/Total			
	RebB	RebA	RebD	RebM
YJR106W			1.38	1.33
YBR043C				1.20
YPR011C				1.27

Table 12. Transport related genes with over a 40% increase in RebA, RebB, RebD or RebM production, compared to a control steviol glycoside-producing strain.

	Increases in Production			
	RebB	RebA	RebD	RebM
YMR166C				1.52
YIL166C		1.41	1.50	1.55
YKR039W			1.48	1.52
YKL146W				1.42
YJL093C			1.46	1.43
YOR306C				1.59
YDL128W				1.49
YOL122C			1.41	1.59
YIL006W			1.64	2.03
YFL028C				1.55
YBR021W			1.51	1.87
YHR002W			1.51	1.73
YEL031W			1.45	1.66
YCL069W				1.53
YOL158C			1.42	1.63
YKL064W			1.40	1.44
YHR096C				1.42

	Increases in Production			
	RebB	RebA	RebD	RebM
YOR332W				1.44
YDR338C			1.50	1.55
YJR106W			1.41	1.44
YBR043C			1.55	1.49
YPR011C				1.43
YFR045W			1.44	

Example 6. Genomic Integration of Transporter Genes

[00161] DNA of the transporter genes selected for integration into the genome of a RebD/M-producing *S. cerevisiae* strain (see Example 2) was amplified from an S288C background by PCR and cloned into a plasmid with homology regions for the integration site and a PGK1 promoter for overexpression, using the USER cloning system. See, e.g., Nour-Eldin *et al.*, 2010, Methods Mol Biol. 643:185-200. The USER cloning construct including the homology regions and the transporter was cut out from the plasmid using restriction enzymes, and the linear piece of DNA was integrated into the genome of the receiving RebD/M-producing strain by standard LiAc method. The genomically integrated transporters were tested in plates that release glucose from a polymer after addition of a growth medium. A polymer that releases 20 g/L glucose over 3 days was used to mimic the feed profile during fermentation. Steviol glycoside levels were measured by LC-MS (see Example 1), and OD₆₀₀ was measured on a Perkin Elmer 2104 Multilabel reader. YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), YJL093C (SEQ ID NO:44), YJR106W (SEQ ID NO:48), YKL120W (SEQ ID NO:126), and YMR166C (SEQ ID NO:132) showed improved excretion of 13-SMG. (Figure 4A). YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), and YMR166C (SEQ ID NO:132) showed improved excretion of RebA (Figure 4B). YBR043C (SEQ ID NO:88), YEL027W (SEQ ID NO:102), and YMR166C (SEQ ID NO:132) showed improved excretion of RebB (Figure 4C). YBR043C of SEQ ID NO:88, YEL027W of SEQ ID NO:102, YJL093C of SEQ ID NO:44, YJR106W of SEQ ID NO:48, and YMR166C of SEQ ID NO:132 showed improved production of RebD, and YBR043C of SEQ ID NO:88, YEL027W of SEQ ID NO:102, YIL166C (SEQ ID NO:121), YJL093C of SEQ ID NO:44, YJR106W of SEQ ID NO:48, and YMR166C of SEQ ID NO:132 showed improved production of RebM, as measured by an increase in RebD and RebM levels in the

supernatant compared to a control steviol glycoside-producing strain. See Figures 4D and 4E. Controls with a URA marker are also shown in Figure 4.

[00162] Figure 5A shows supernatant levels of RebA, RebB, RebD, and RebM of an additional steviol glycoside-producing strain overexpressing YMR166C (SEQ ID NO:132), YEL027W (SEQ ID NO:102), YKL120W (SEQ ID NO:126), YJR106W (SEQ ID NO:48), YJL093C (SEQ ID NO:44), and YBR043C (SEQ ID NO:88) by the USER cloning system. The strain of Figure 5 comprised a recombinant gene encoding a *Synechococcus* sp. GGPPS polypeptide (SEQ ID NO:1, SEQ ID NO:149), a recombinant gene encoding a truncated *Zea mays* CDPS polypeptide (SEQ ID NO:2, SEQ ID NO:150), a recombinant gene encoding an *A. thaliana* KS polypeptide (SEQ ID NO:3, SEQ ID NO:151), a recombinant gene encoding a recombinant *S. rebaudiana* KO1 polypeptide (SEQ ID NO:4, SEQ ID NO:152), a recombinant gene encoding a KO polypeptide (SEQ ID NO:XX, SEQ ID NO:XX), a recombinant gene encoding an *A. thaliana* ATR2 polypeptide (SEQ ID NO:5, SEQ ID NO:153), a recombinant gene encoding an *O. sativa* EUGT11 polypeptide (SEQ ID NO:12; SEQ ID NO:148), a recombinant gene encoding an SrKAHe1 polypeptide (SEQ ID NO:6, SEQ ID NO:154), a recombinant gene encoding an *S. rebaudiana* CPR8 polypeptide (SEQ ID NO:7, SEQ ID NO:155), a recombinant gene encoding an *S. rebaudiana* UGT85C2 polypeptide (SEQ ID NO:8, SEQ ID NO:156), a recombinant gene encoding an *S. rebaudiana* UGT74G1 polypeptide (SEQ ID NO:9, SEQ ID NO:157), a recombinant gene encoding an *S. rebaudiana* UGT76G1 polypeptide (SEQ ID NO:10, SEQ ID NO:158), and a recombinant gene encoding an *S. rebaudiana* UGT91D2 variant (or functional homolog), UGT91D2e-b (SEQ ID NO:11, SEQ ID NO:159) polypeptide. Figure 5B shows total levels of RebA, RebB, RebD, and RebM of the above described steviol glycoside-producing strain overexpressing YMR166C (SEQ ID NO:132), YEL027W (SEQ ID NO:102), YKL120W (SEQ ID NO:126), YIL166C (SEQ ID NO:132), YJR106W (SEQ ID NO:48), YJL093C (SEQ ID NO:44), and YBR043C (SEQ ID NO:88) by the USER cloning system.

Example 7. Production of RebD and RebM by Fermentation of Steviol Glycoside-Producing *S. cerevisiae* strains overexpressing YJL093C or YBR043C

[00163] YJL093C (SEQ ID NO:44) and YBR043C (SEQ ID NO:88) were individually overexpressed in the steviol glycoside-producing strain described in Example 3. The strains were cultivated by fermentation (fed-batch, minimum medium, glucose-limiting) for approximately 130 h. Production of RebD and RebM was measured by LC-MS. As shown in Table 13, the strains overexpressing YJL093C or YBR043C produced higher levels of RebD and RebD + RebM, as compared to a control steviol glycoside-producing strain.

Table 13. Production of RebD and RebM in *S. cerevisiae* strains overexpressing YJL093C and YBR043C.

Strain	Ferm. Length (h)	Final Cell Dry Weight	RebD Titer (g/L)	RebM Titer (g/L)	RebD + RebM	RebD/Reb M Ratio (g/g)
Control	126.83	104.53	1.38	4.47	5.85	0.31
YJL093C	130.10	114.40	3.42	2.80	6.22	1.22
YBR043C	129.17	112.00	3.56	2.72	6.28	1.31

Table 14. Sequences disclosed herein.

SEQ ID NO:1

Synechococcus sp. GGPPS (GenBank ABC98596.1)

atgggtcgac	aaactttcaa	cctggatacc	tacttatccc	aaagacaaca	acaagttgaa	60
gaggccctaa	gtgctgctct	tgtgccagct	tatcctgaga	gaatatacga	agctatgaga	120
tactccctcc	tggcaggtgg	caaaagatta	agacctatct	tatgttttagc	tgcttgcgaa	180
ttggcaggtg	gttctgttga	acaagccatg	ccaactgcgt	gtgcacttga	aatgatccat	240
acaatgtcac	taattcatga	tgacctgcca	gccatggata	acgatgattt	cagaagagga	300
aagccaacta	atcacaaggt	gttcggggaa	gatatagcca	tcttagcggg	tgatgcgctt	360
ttagcttacg	cttttgaaca	tattgcttct	caaacaagag	gagtagccacc	tcaattggtg	420
ctacaagtta	ttgctagaat	cggacacgcc	gttgctgcaa	caggcctcgt	tggaggccaa	480
gtcgtagacc	ttgaatctga	aggtaaagct	atttccttag	aaacattgga	gtatattcac	540
tcacataaga	ctggagcctt	gctggaagca	tcagttgtct	caggcggtat	tctcgcaggg	600
gcagatgaag	agcttttggc	cagattgtct	cattacgcta	gagatatagg	cttggctttt	660
caaatcgtcg	atgatatcct	ggatgttact	gctacatctg	aacagttggg	gaaaaccgct	720
ggtaaagacc	aggcagccgc	aaaggcaact	tatccaagtc	tattgggttt	agaagcctct	780
agacagaaag	cggaagagtt	gattcaatct	gctaaggaag	ccttaagacc	ttacggttca	840
caagcagagc	cactcctagc	gctggcagac	ttcatcacac	gtcgtcagca	ttaa	894

SEQ ID NO:2

Zea mays truncated CDPS

atggcacagcaca	catcagaatc	cgcagctgtc	gcaaagggca	gcagtttgac	ccctatagtg	60
agaactgacg	ctgagtcaag	gagaacaaga	tggccaaccg	atgacgatga	cgccgaacct	120
ttagtggatg	agatcagggc	aatgcttact	tccatgtctg	atggtgacat	ttccgtgagc	180
gcatacgata	cagcctgggt	cggattgggt	ccaagattag	acggcgggtga	aggtcctcaa	240
tttccagcag	ctgtgagatg	gataagaaat	aaccagttgc	ctgacggaag	ttggggcgat	300
gccgcattat	tctctgccta	tgacaggctt	atcaataccc	ttgcctgcgt	tgtaactttg	360
acaaggtggg	ccctagaacc	agagatgaga	ggtagaggac	tatctttttt	gggtaggaac	420
atgtggaaat	tagcaactga	agatgaagag	tcaatgccta	ttggcttcga	attagcattt	480
ccatctttga	tagagcttgc	taagagccta	ggtgtccatg	acttccctta	tgatcaccag	540
gccctacaag	gaatctactc	ttcaagagag	atcaaaatga	agaggattcc	aaaagaagtg	600
atgcataccg	ttccaacatc	aatattgcac	agtttggagg	gtatgcctgg	cctagattgg	660
gctaaactac	ttaaactaca	gagcagcgac	ggaagttttt	tgttctcacc	agctgccact	720
gcatatgctt	taatgaatac	cggagatgac	aggtgtttta	gctacatcga	tagaacagta	780
aagaaattca	acggcggcgt	ccctaattgtt	tatccagtgg	atctatttga	acatatttgg	840
gccgttgata	gacttgaaag	attaggaatc	tccagggtact	tccaaaagga	gatcgaacaa	900
tgcatggatt	atgtaaacag	gcattggact	gaggacggta	tttgttgggc	aaggaactct	960
gatgtcaaag	aggtggacga	cacagctatg	gccttttagac	ttcttaggtt	gcacggctac	1020
agcgtcagtc	ctgatgtgtt	taaaaacttc	gaaaaggacg	gtgaattttt	cgcatttgtc	1080
ggacagtcta	atcaagctgt	taccggtatg	tacaacttaa	acagagcaag	ccagatatcc	1140
ttcccaggcg	aggatgtgct	tcatagagct	ggtgccttct	catatgagtt	cttgaggaga	1200
aaagaagcag	aggagcctt	gagggacaag	tggatcattt	ctaaagatct	acctggtgaa	1260
gttgtgtata	cttttgattt	tccatgggtac	ggcaacttac	ctagagtcga	ggccagagac	1320
tacctagagc	aatacggagg	tggatgatgac	gtttggattg	gcaagacatt	gtataggatg	1380
ccacttgtaa	acaatgatgt	atatttggaa	ttggcaagaa	tggatttcaa	ccactgccag	1440
gctttgcatc	agtttagagt	gcaaggacta	aaaagatggg	atactgaaaa	taggttgatg	1500
gactttgggtg	tgcCCAAGA	agatgccctt	agagcttatt	ttcttgcagc	cgcattctgtt	1560
tacgagcctt	gtagagctgc	cgagaggctt	gcatgggcta	gagccgcaat	actagctaac	1620
gccgtgagca	cccacttaag	aaatagccca	tcattcagag	aaaggttaga	gcattctctt	1680
aggtgtagac	ctagtgaaga	gacagatggc	tcctggttta	actcctcaag	tggctctgat	1740
gcagtttttag	taaaggctgt	cttaagactt	actgattcat	tagccaggga	agcacagcca	1800
atccatggag	gtgacccaga	agatattata	cacaagttgt	taagatctgc	ttgggccgag	1860

tgggttaggg	aaaaggcaga	cgctgccgat	agcgtgtgca	atggtagttc	tgcagtagaa	1920
caagagggat	caagaatggg	ccatgataaa	cagacctgtc	tattattggc	tagaatgatc	1980
gaaatttctg	ccggtagggc	agctgggtgaa	gcagccagtg	aggacggcga	tagaagaata	2040
attcaattaa	caggctccat	ctgcgacagt	cttaagcaaa	aaatgctagt	ttcacaggac	2100
cctgaaaaaa	atgaagagat	gatgtctcac	gtggatgacg	aattgaagtt	gaggattaga	2160
gagttcgttc	aatatattgct	tagactaggt	gaaaaaaaga	ctggatctag	cgaaaccagg	2220
caaacatttt	taagtatagt	gaaatcatgt	tactatgctg	ctcattgccc	acctcatgtc	2280
gttgatagac	acattagtag	agtgattttc	gagccagtaa	gtgccgcaaa	gtaaccgcgg	2340

SEQ ID NO:3

Arabidopsis thaliana KS (similar to GenBank AEE36246.1)

atgtctatta	atttgagatc	ttccggttgt	agctcccca	taagcgcaac	tttggaaagg	60
ggtctagact	ctgaagttca	aacaagagca	aacaatgtat	cttttgagca	gaccaaagag	120
aagatcagga	aaatgcttga	gaaggctcga	ttgagcgtga	gtgcctatga	cactagttgg	180
gtagctatgg	tcccatcacc	atccagtcaa	aacgcacctc	ttttcccaca	gtgcgtcaaa	240
tggctacttg	ataatcaaca	tgaggacggc	tcttggggat	tggataacca	cgaccatcag	300
agcttaaaga	aagatgtggt	gtcatccaca	ttagcctcta	tcctagctct	taagaaatgg	360
ggaataggcg	aaagacagat	caataagggt	ctacagttca	ttgaattaaa	ctctgcacta	420
gttaccgatg	aaactataca	aaaacctaca	ggtttcgaca	tcatttttcc	aggaatgatt	480
aagtacgcca	gggaccttaa	tttgaccata	cctcttggtc	cagaagtagt	cgacgatatg	540
atcaggaaaa	gagatctaga	cttaaagtgt	gatagcgaga	aattcagcaa	aggtagagag	600
gcttatcttg	cctatgttct	tgaaggaact	aggaacttga	aggactggga	cttaattgtg	660
aaatatcaga	gaaagaacgg	tagtctattt	gatagtccag	ctacaaccgc	cgcagctttc	720
actcaatttg	gcaatgacgg	ttgcttgagg	tacttatggt	cacttttaca	gaaattcgag	780
gccgcagtgc	ctagtgtata	tccatttgat	caatacgcta	gattaagcat	aatcgtcact	840
ttagaatcat	tgggaattga	cagagatttc	aagactgaga	taaaaagcat	attggatgag	900
acctataggt	actggcttag	aggtgacgaa	gaaatttgcc	tagatttggc	cacatgtgca	960
cttgctttta	ggttgctttt	agcccacggc	tatgacgtgt	catacgatcc	tctaaagcca	1020
tttgagagag	aatctgggtt	cagcgatacc	cttgagggat	atgttaaaaa	caccttttcc	1080
gtattagagc	ttttcaaggc	tgcccaaagt	taccctcatg	agagtgcctt	gaaaaagcag	1140
tgttgctgga	caaaacaata	tctagaaatg	gaactaagtt	catgggttaa	aacaagcggt	1200
agggacaagt	acttgaaaaa	ggaagtggag	gatgcttttg	catttccatc	atatgcctct	1260
ttagaaagaa	gtgaccacag	aaggaaaatt	cttaatggct	cagcagttga	aaacacaaga	1320
gtaaccaaga	cctcttacag	gttgcataat	atatgtacat	cagatatctt	aaaacttgct	1380
gtcgacgatt	tcaacttttg	ccaatctatt	catagagagg	aaatggaaag	attggataga	1440
tggatagtgg	agaatagact	acaggaatta	aagttcgcca	gacaaaaatt	ggcttactgt	1500
tacttttagtg	gcgctgccac	actattctct	ccagaattgt	ctgacgcaag	gatctcatgg	1560
gctaagggag	gtgttctaac	cacagtagtc	gatgactttt	ttgatgttgg	cggtagtaaa	1620
gaagagcttg	agaacttaat	tcacttggtg	gaaaagtggg	atcttaatgg	agttcctgaa	1680
tactcttcag	agcatgtaga	aataattttc	tctgtcctaa	gagacactat	cttagaaacc	1740
ggtgataaag	cctttacata	tcagggcaga	aacgttactc	accatattgt	gaaaatatgg	1800
ttggacttac	ttaagagcat	gctaagggag	gctgaatggt	ccagtgacaa	atcaacccca	1860
tctttggaag	attacatgga	gaatgcctat	atcagcttcg	cattaggtcc	tattgtattg	1920
ccagctacat	accttatagg	acctccacta	cctgaaaaga	ctgtcgactc	ccaccaatat	1980
aatcaattat	acaaattggg	tagtaccatg	ggtagactat	taaacgatat	ccagggtctt	2040
aagagggaat	cagccgaggg	aaaacttaat	gcagtgtctc	tacatatgaa	gcatgaaaga	2100
gacaacagaa	gcaaagaggt	tattatagaa	tccatgaaag	gattggctga	aaggaaaaga	2160
gaggaattac	acaaacttgt	actagaagag	aaaggtagtg	tcgttccaag	agaatgcaag	2220
gaagccttct	taaaaatgtc	aaaagtgttg	aacctttttt	ataggaagga	tgatggcttc	2280
acatctaacg	acttgatgag	ccttgtgaaa	tccgtcatct	acgagcctgt	ttcacttcaa	2340
aaggagagtc	taacttga					2358

SEQ ID NO:4

S. rebaudiana KO1 (codon optimized)

atggatgctg	tgacggggtt	gttaactgtc	ccagcaaccg	ctataactat	tggtggaact	60
gctgtagcat	tggcggtagc	gctaattctt	tggtacctga	aatcctacac	atcagctaga	120
agatcccaat	caaatcatct	tccaagagtg	cctgaagtcc	caggtgttcc	attgttagga	180
aatctgttac	aattgaagga	gaaaaagcca	tacatgactt	ttacgagatg	ggcagcgaca	240
tatggacct	tctatagtat	caaaactggg	gctacaagta	tggttgtggt	atcatcta	300
gagatagcca	aggaggcatt	ggtgaccaga	ttccaatcca	tatctacaag	gaacttatct	360
aaagccctga	aagtacttac	agcagataag	acaatggctg	caatgtcaga	ttatgatgat	420
tatcataaaa	cagttaagag	acacatactg	accgccgtct	tggttcctaa	tgcacagaaa	480
aagcatagaa	ttcacagaga	tatcatgatg	gataacatat	ctactcaact	tcatgaattc	540
gtgaaaaaca	accagaaca	ggaagaggta	gaccttagaa	aaatctttca	atctgagtta	600
ttcggcttag	ctatgagaca	agccttagga	aaggatgttg	aaagtttgta	cgttgaagac	660
ctgaaaatca	ctatgaatag	agacgaaatc	tttcaagtcc	ttgttggtga	tccaatgatg	720
ggagcaatcg	atgttgattg	gagagacttc	tttccatacc	taaagtgggt	cccaaacaaa	780
aagttcgaaa	atactattca	acaaatgtac	atcagaagag	aagctgttat	gaaatcttta	840
atcaaagagc	acaaaaagag	aatagcgtca	ggcgaaaagc	taaatagtta	tatcgattac	900
cttttatctg	aagctcaaac	tttaaccgat	cagcaactat	tgatgtcctt	gtgggaacca	960
atcattgaat	cttcagatac	aacaatggtc	acaacagaat	gggcaatgta	cgaattagct	1020
aaaaacccta	aattgcaaga	taggttgtag	agagacatta	agtcctgtct	tggtctgaa	1080
aagataaccg	aagagcatct	atcacagctg	ccttacatta	cagctatttt	ccacgaaaca	1140
ctgagaagac	actcaccagt	tcctatcatt	cctctaagac	atgtacatga	agataccggt	1200
ctaggcggct	accatgttcc	tgctggcaca	gaacttgccg	ttaacatcta	cggttgcaac	1260
atggacaaaa	acgtttggga	aaatccagag	gaatggaacc	cagaaagatt	catgaaagag	1320
aatgagacaa	ttgattttca	aaagacgatg	gccttcgggtg	gtggtaagag	agtttgtgct	1380
ggttccttgc	aagccctttt	aactgcatct	attgggattg	ggagaatggg	tcaagagttc	1440
gaatggaaac	tgaaggatat	gactcaagag	gaagtgaaca	cgataggcct	aactacacaa	1500
atgttaagac	cattgagagc	tattatcaaa	cctaggatct	aa		1542

SEQ ID NO:5

A. thaliana ATR2 (codon optimized)

atgtcttcct	cttcctcttc	cagtacctct	atgattgatt	tgatggctgc	tattattaaa	60
ggtgaaccag	ttatcgtctc	cgaccagca	aatgcctctg	cttatgaatc	agttgctgca	120
gaattgtctt	caatgttgat	cgaaaacaga	caattcgcca	tgatcgtaac	tacatcaatc	180
gctgttttga	tcggttgat	tgatcatgtt	gtatggagaa	gatccggtag	tggttaattct	240
aaaagagtcg	aacctttgaa	accattagta	attaagccaa	gagaagaaga	aatagatgac	300
ggtagaaaaga	aagttacaat	atttttcggg	acccaaactg	gtacagctga	aggttttgca	360
aaagccttag	gtgaagaagc	taaggcaaga	tacgaaaaga	ctagattcaa	gatagtcgat	420
ttggatgact	atgccgctga	tgacgatgaa	tacgaagaaa	agttgaagaa	agaagatggt	480
gcatttttct	ttttggcaac	ctatgggtgac	ggtgaaccaa	ctgacaatgc	agccagattc	540
tacaaatggg	ttacagaggg	taatgatcgt	ggtgaatggg	tgaaaaactt	aaagtacggg	600
gttttcgggt	tggttaacag	acaatacgaa	catttcaaca	aagttgcaaa	ggttgtcgac	660
gatatttttg	tcgaacaagg	tgctcaaaga	ttagtccaag	taggtttggg	tgacgatgac	720
caatgtatag	aagatgactt	tactgcctgg	agagaagcct	tgtggcctga	attagacaca	780
atcttgagag	aagaagggtga	caccgccgtt	gctaccccat	atactgctgc	agtattagaa	840
tacagagttt	ccatccatga	tagtgaagac	gcaaagttta	atgatatcac	tttggccaat	900
ggtaacgggt	atacagtttt	cgatgcacaa	cacccttaca	aagctaactg	tgcaagtcaag	960
agagaattac	atacaccaga	atccgacaga	agttgtatac	acttggaatt	tgatatcgct	1020
ggttccgggt	taaccatgaa	gttgggtgac	catgtagggt	ttttatgcga	caatttgtct	1080
gaaactgttg	atgaagcatt	gagattgttg	gatatgtccc	ctgacactta	ttttagtttg	1140
cacgctgaaa	aagaagatgg	tacaccaatt	tccagttctt	taccacctcc	attccctcca	1200
tgtaacttaa	gaacagcctt	gaccagatac	gottgcttgt	tatcatcccc	taaaaagtcc	1260
gccttggttg	cttttagccg	tcatgctagt	gacctactg	aagcagaaag	attgaaacac	1320
ttagcatctc	cagccggtaa	agatgaatat	tcaaagtggg	tagttgaatc	tcaaagatca	1380

ttgttagaag	ttatggcaga	atttccatct	gccaagcctc	cattaggtgt	cttctttgct	1440
ggtgtagcac	ctagattgca	accaagattc	tactcaatca	gttcttcacc	taagatcgct	1500
gaaactagaa	ttcatgttac	atgtgcatta	gtctacgaaa	agatgccaac	cggtagaatt	1560
cacaaggggtg	tatgctctac	ttggatgaaa	aatgctgttc	cttacgaaaa	atcagaaaag	1620
ttgttcttag	gtagaccaat	cttcgtaaga	caatcaaact	tcaagttgcc	ttctgattca	1680
aaggttccaa	taatcatgat	aggtcctggt	acaggtttag	ccccattcag	aggtttcttg	1740
caagaaagat	tggcttttagt	tgaatctggt	gtcgaattag	gtccttcagt	tttgttcttt	1800
ggttgtagaa	acagaagaat	ggatttcatc	tatgaagaag	aattgcaaag	attcgtcgaa	1860
tctggtgcat	tggccgaatt	atctgtagct	ttttcaagag	aagggtccaac	taaggaatac	1920
gttcaacata	agatgatgga	taaggcatcc	gacatatgga	acatgatcag	tcaaggtgct	1980
tatttgtacg	tttgcggtga	cgcaaagggg	atggccagag	atgtccatag	atctttgcac	2040
acaattgctc	aagaacaagg	ttccatggat	agtaccaaag	ctgaagggtt	cgtaaagaac	2100
ttacaaactt	ccggtagata	cttgagagat	gtctgggtga			2139

SEQ ID NO:6

Stevia rebaudiana KAHel (codon-optimized)

atggaagcct	cttacctata	catttctatt	ttgctttttac	tggcatcata	cctgttcacc	60
actcaactta	gaaggaagag	cgctaatacta	ccaccaaccg	tgtttccatc	aataccaatc	120
attggacact	tatacttact	caaaaagcct	ctttatagaa	cttttagcaa	aattgccgct	180
aagtacggac	caatactgca	attacaactc	ggctacagac	gtgttctggt	gatttcctca	240
ccatcagcag	cagaagagt	ctttaccaat	aacgatgtaa	tcttcgcaa	tagacctaat	300
acattgtttg	gcaaaatagt	gggtggaaca	tcccttggca	gtttatccta	cggcgatcaa	360
tggcgtaatc	taaggagagt	agcttctatc	gaaatcctat	cagttcatag	gttgaacgaa	420
tttcatgata	tcagagtgga	tgagaacaga	ttgttaatta	gaaaacttag	aagttcatct	480
tctcctgtta	ctcttataac	agtctttttat	gctctaacat	tgaacgtcat	tatgagaatg	540
atctctggca	aaagatat	cgacagtggg	gatagagaat	tggaggagga	aggtaagaga	600
tttcgagaaa	tcttagacga	aacgttgctt	ctagccggtg	cttctaata	tggcgactac	660
ttaccaatat	tgaactgggt	gggagttaag	tctcttgaaa	agaaattgat	cgttttgcag	720
aaaaagagag	atgacttttt	ccagggtttg	attgaacagg	ttagaaaatc	tcgtgggtgct	780
aaagtaggca	aaggtagaaa	aacgatgatc	gaactcttat	tatctttgca	agagtcagaa	840
cctgagtact	atacagatgc	tatgataaga	tcttttgtcc	taggtctgct	ggctgcaggt	900
agtgatactt	cagcgggcac	tatggaatgg	gccatgagct	tactgggtcaa	tcacccacat	960
gtattgaaga	aagctcaagc	tgaaatcgat	agagttatcg	gtaataacag	attgattgac	1020
gagtcagaca	ttggaaatat	cccttacatc	gggtgtatta	tcaatgaaac	tctaagactc	1080
tatccagcag	ggccattggt	gttcccacat	gaaagtctctg	ccgactgcgt	tatttccggt	1140
tacaatat	ctagagggtac	aatgttaatc	gtaaaccaat	gggcgattca	tcacgatcct	1200
aaagtctggg	atgatcctga	aaccttttaa	cctgaaagat	ttcaaggatt	agaaggaact	1260
agagatgggt	tcaaacttat	gccattcggt	tctgggagaa	gaggatgtcc	agggtgaagg	1320
ttggcaataa	ggctgttagg	gatgacacta	ggctcagtga	tccaatgttt	tgattgggag	1380
agagtaggag	atgagatggt	tgacatgaca	gaaggtttgg	gtgtcacact	tcctaaggcc	1440
gttccattag	ttgccaaatg	taagccacgt	tccgaaatga	ctaattctct	atccgaactt	1500
taa						1503

SEQ ID NO:7

Stevia rebaudiana CPR8

ATGCAATCTAACTCCGTGAAGATTTGCGCCGCTTGATCTGGTAACTGCGCTGTTTAGCGGCAAGGTTTT
GGACACATCGAACGCATCGGAATCGGGAGAATCTGCTATGCTGCCGACTATAGCGATGATTATGGAGA
ATCGTGAGCTGTTGATGATACTCACAACGTCGGTTGCTGTATTGATCGGATGCGTTGTCGTTTTGGTG
TGGCGGAGATCGTCTACGAAGAAGTCGGCGTTGGAGCCACCGGTGATTGTGGTTCCGAAGAGAGTGCA
AGAGGAGGAAGTTGATGATGGTAAGAAGAAAGTTACGGTTTTCTTCGGCACCCAAACTGGAACAGCTG
AAGGCTTCGCTAAGGCACTTGTTGAGGAAGCTAAAGCTCGATATGAAAAGGCTGTCTTTAAAGTAATT
GATTTGGATGATTATGCTGCTGATGACGATGAGTATGAGGAGAACTAAAGAAAGAATCTTTGGCCTT

TTTCTTTTTGGCTACGTATGGAGATGGTGAGCCAACAGATAATGCTGCCAGATTTTATAAATGGTTTA
 CTGAGGGAGATGCGAAAGGAGAATGGCTTAATAAGCTTCAATATGGAGTATTTGGTTTGGGTAACAGA
 CAATATGAACATTTTAACAAGATCGCAAAAGTGGTTGATGATGGTCTTGTAGAACAGGGTGCAAAGCG
 TCTTGTTCCCTGTTGGACTTGGAGATGATGATCAATGTATTGAAGATGACTTCACCGCATGGAAAGAGT
 TAGTATGGCCGGAGTTGGATCAATTACTTCGTGATGAGGATGACACAACCTGTTGCTACTCCATACACA
 GCTGCTGTTGCAGAATATCGCGTTGTTTTTTCATGAAAAACCAGACGCGCTTTCTGAAGATTATAGTTA
 TACAAATGGCCATGCTGTTTCATGATGCTCAACATCCATGCAGATCCAACGTGGCTGTCAAAAAGGAAC
 TTCATAGTCCTGAATCTGACCGGTCTTGCACTCATCTTGAATTTGACATCTCGAACACCGGACTATCA
 TATGAAACTGGGGACCATGTTGGAGTTTACTGTGAAAACCTTGAGTGAAGTTGTGAATGATGCTGAAAG
 ATTAGTAGGATTACCACCAGACACTTACTCCTCCATCCACACTGATAGTGAAGACGGGTCGCCACTTG
 GCGGAGCCTCATTGCCGCCTCCTTTCCCGCCATGCACTTTAAGGAAAGCATTGACGTGTTATGCTGAT
 GTTTTGAGTTCTCCCAAGAAGTCGGCTTTGCTTGCACTAGCTGCTCATGCCACCGATCCCAGTGAAGC
 TGATAGATTGAAATTTCTTGTCATCCCCCGCCGGAAGGATGAATATTCTCAATGGATAGTTGCAAGCC
 AAAGAAGTCTCCTTGAAGTCATGGAAGCATTCCCGTCAGCTAAGCCTTCACTTGGTGTTTTCTTTGCA
 TCTGTTGCCCCGCGCTTACAACCAAGATACTACTCTATTTCTTCCTCACCCAAGATGGCACCGGATAG
 GATTCATGTTACATGTGCATTAGTCTATGAGAAAACACCTGCAGGCCGCATCCACAAAGGAGTTTGTT
 CAACTTGGATGAAGAACGCAGTGCCTATGACCGAGAGTCAAGATTGCAGTTGGGCCCCAATATACGTC
 CGAACATCCAATTTTCAGACTACCATCTGACCCTAAGGTCCCGGTTATCATGATTGGACCTGGCACTGG
 TTTGGCTCCTTTTAGAGGTTTCCTTCAAGAGCGGTTAGCTTTAAAGGAAGCCGGAAGTACCTCGGTT
 TATCCATTTTATTCTTCGGATGTAGGAATCGCAAAGTGGATTTTCATATATGAAAACGAGCTTAACAAC
 TTTGTGGAGACTGGTGCTCTTTCTGAGCTTATTGTTGCTTTCTCCCGTGAAGGCCCGACTAAGGAATA
 TGTGCAACACAAGATGAGTGAGAAGGCTTCGGATATCTGGAACCTTGCTTTCTGAAGGAGCATATTTAT
 ACGTATGTGGTGATGCCAAAGGCATGGCCAAAGATGTACATCGAACCCTCCACACAATTGTGCAAGAA
 CAGGGATCTCTTGACTCGTCAAAGGCAGAACTCTACGTGAAGAATCTACAAATGTCAGGAAGATACCT
 CCGTGACGTTTGGTAA

SEQ ID NO:8

Stevia rebaudiana UGT85C2 (codon optimized)

atggatgcaa tggcaactac tgagaaaaag cctcatgtga tcttcattcc atttcctgca	60
caatctcaca taaaggcaat gctaaagtta gcacaactat tacaccataa gggattacag	120
ataactttcg tgaataccga cttcatccat aatcaatttc tggaatctag tggccctcat	180
tgtttggaag gagccccagg gttagatttc gaaacaattc ctgacggtgt ttcacattcc	240
ccagaggcct ccatcccaat aagagagagt ttactgaggt caatagaaac caactttttg	300
gatcgtttca ttgacttggt cacaaaactt ccagaccac caacttgcac aatctctgat	360
ggctttctgt cagtgtttac tatcgacgct gccaaaaagt tgggtatccc agttatgatg	420
tactggactc ttgctgcatg cggtttcatg ggtttctatc acatccattc tcttatcgaa	480
aagggttttg ctccactgaa agatgcatca tacttaacca acggctacct ggatactgtt	540
attgactggg taccaggtat ggaaggtata agacttaaag attttccttt ggattggtct	600
acagacctta atgataaagt attgatgttt actacagaag ctccacaaag atctcataag	660
gtttcacatc atatctttca cacctttgat gaattggaac catcaatcat caaaaccttg	720
tctctaagat acaatcatat ctacactatt ggtccattac aattacttct agatcaaatt	780
cctgaagaga aaaagcaaac tggatttaca tccttacacg gctactcttt agtgaaagag	840
gaaccagaat gttttcaatg gctacaaagt aaagagccta attctgtggt ctacgtcaac	900
ttcggaagta caacagtcac gtccttgga gatatgactg aatttggttg gggccttgct	960
aattcaaadc attactttct atggattatc aggtccaatt tggtaatagg ggaaaacgcc	1020
gtattacctc cagaattgga ggaacacatc aaaaagagag gtttcattgc ttcctggtgt	1080
tctcaggaaa aggtattgaa acatccttct gttggtggtt tccttactca ttgcggttgg	1140
ggctctacaa tcgaatcact aagtgcagga gttccaatga tttgttggcc atattcatgg	1200
gaccaactta caaattgtag gtatatctgt aaagagtggg aagttggatt agaaatggga	1260
acaaagggtta aacgtgatga agtgaaaaga ttggttcagg agttgatggg ggaagggtggc	1320

cacaagatga	gaaacaaggc	caaagattgg	aaggaaaaag	ccagaattgc	tattgctcct	1380
aacgggtcat	cctctctaaa	cattgataag	atgggtcaaag	agattacagt	cttagccaga	1440
aactaa						1446

SEQ ID NO:9

S. rebaudiana UGT74G1 (GenBank AAR06920.1)

atggcggaac	aacaaaagat	caagaaatca	ccacacgttc	tactcatccc	attcccttta	60
caaggccata	taaacccttt	catccagttt	ggcaaacgat	taatctccaa	aggtgtcaaa	120
acaacacttg	ttaccaccat	ccacacctta	aactcaacc	taaaccacag	taacaccacc	180
accacctcca	tcgaaatcca	agcaatttcc	gatggttgtg	atgaaggcgg	ttttatgagt	240
gcaggagaat	catatttgga	aacattcaaa	caagttgggt	ctaaatcact	agctgactta	300
atcaagaagc	ttcaaagtga	aggaaccaca	attgatgcaa	tcatttatga	ttctatgact	360
gaatggggtt	tagatgttgc	aattgagttt	ggaatcgatg	gtggttcgtt	tttcactcaa	420
gcttgtgttg	taaacagctt	atattatcat	gttcataagg	gtttgatttc	tttgccattg	480
ggtgaaactg	tttcggttcc	tggatttcca	gtgcttcaac	gggtgggagac	accgttaatt	540
ttgcagaatc	atgagcaa	acagagccct	tgggtctcaga	tggtgtttgg	tcagtttgct	600
aatattgatc	aagcacgttg	ggtcttcaca	aatagttttt	acaagctcga	ggaagaggta	660
atagagtggg	cgagaaagat	atggaacttg	aaggtaatcg	ggccaacact	tccatccatg	720
taccttgaca	aacgacttga	tgatgataaa	gataacggat	ttaatctcta	caaagcaa	780
catcatgagt	gcatgaactg	gttagacgat	aagccaaagg	aatcagttgt	ttacgtagca	840
tttggtagcc	tggtgaaaca	tggacccgaa	caagtgggag	aatcacacg	ggctttaata	900
gatagtgatg	tcaacttctt	gtgggttatc	aaacataaag	aagaggggaa	gctcccagaa	960
aatctttcgg	aagtaataaa	aaccgggaaag	ggtttgattg	tagcatgggtg	caaacaattg	1020
gatgtgttag	cacacgaatc	agtaggatgc	tttgttacac	attgtgggtt	caactcaact	1080
cttgaagcaa	taagtcttgg	agtccccggt	gttgcaatgc	ctcaattttc	ggatcaaact	1140
acaaatgcca	agcttctaga	tgaatttttg	ggtgttgagg	ttagagttaa	ggctgatgag	1200
aatgggatag	tgagaagagg	aatcttgcg	tcatgtatta	agatgattat	ggaggaggaa	1260
agaggagtaa	taatccgaaa	gaatgcggta	aatggaagg	atttggttaa	agtagccgtt	1320
catgaagggtg	gtagctcaga	caatgatatt	gtcgaatttg	taagtgagct	aattaaggct	1380
taaatttttg	ttgctttgta	ttttatgtgt	tatggttttt	tgatttagat	gtattcaatt	1440
aatattgaat	cataactaaa	ttcaagatta	ttgtttgtaa	tattctttgt	cctaaaattt	1500
tgcgacttaa	aacctttagt	ttataaaaag	aaattagaaa	atactattgc	acgga	1555

SEQ ID NO:10

S. rebaudiana UGT76G1 (codon optimized)

atggaaaaca	agaccgaaac	aacagttaga	cgtaggcgta	gaatcattct	gtttccagta	60
ccttttcaag	ggcacatcaa	tccaatacta	caactagcca	acgttttgta	ctctaaaggt	120
ttttctatta	caatctttca	caccaatttc	aacaaaccaa	aaacatccaa	ttaccacat	180
ttcacattca	gattcatact	tgataatgat	ccacaagatg	aacgtatttc	aaacttacct	240
accacgggtc	cttttagctgg	aatgagaatt	ccaatcatca	atgaacatgg	tgccgatgag	300
cttagaagag	aattagagtt	acttatgttg	gcatccgaag	aggacgagga	agtctcttgt	360
ctgattactg	acgctctatg	gtactttgcc	caatctgtgg	ctgatagttt	gaatttgagg	420
agattgggtac	taatgacatc	cagtctgttt	aactttcacg	ctcatgttag	tttaccacaa	480
tttgacgaat	tgggatactt	ggaccctgat	gacaagacta	ggttagagga	acaggcctct	540
ggttttccta	tgttgaaagt	caaagataatc	aagtctgcct	attctaattg	gcaaactctg	600
aaagagatct	taggaaagat	gatcaaacag	acaaaggctt	catctggagt	gatttggaac	660
agtttcaaag	agttagaaga	gtctgaattg	gagactgtaa	tcagagaaat	tccagcacct	720
tcattcctga	taccattacc	aaaacatttg	actgcttctt	cttcctcttt	gttgatcat	780
gacagaacag	tttttcaatg	gttggaacaa	caaccaccta	gttctgtttt	gtacgtgtca	840
tttggtagta	cttctgaagt	cgatgaaaag	gacttccttg	aaatcgcaag	aggcttagtc	900
gatagtaagc	agtcattcct	ttgggtcgtg	cgtccagggt	tcgtgaaagg	ctcaacatgg	960
gtcgaaccac	ttccagatgg	ttttctaggc	gaaagaggta	gaatagtcaa	atgggttcct	1020
caacaggaag	tttttagctca	tggcgctatt	ggggcattct	ggactcattc	cggatggaat	1080

tcaacttttag	aatcagtatg	cgaaggggta	cctatgatct	tttcagattt	tgggtcttgat	1140
caaccactga	acgcaagata	catgtctgat	gttttgaaag	tgggtgtata	tctagaaaat	1200
ggctgggaaa	ggggtgaaat	agctaataca	ataagacgtg	ttatggttga	tgaagagggg	1260
gagtatatca	gacaaaacgc	aagagtgcctg	aagcaaaagg	ccgacgtttc	tctaatagaag	1320
ggaggctctt	catacgaatc	cttagaatct	cttgtttcct	acatttcctc	actgtaa	1377

SEQ ID NO:11

S. rebaudiana UGT91D2e-b (codon optimized)

atggctactt	ctgattccat	cggtgacgat	agaaagcaat	tgcattgttg	tacttttcca	60
tgggtggctt	tcgggtcatat	tttgccatac	ttgcaattgt	ccaagttgat	tgctgaaaag	120
ggtcacaagg	tttcattctt	gtctaccacc	agaaacatcc	aaagattgtc	ctctcatatc	180
tccccattga	tcaacgttgt	tcaattgact	ttgccaagag	tccaagaatt	gccagaagat	240
gctgaagcta	ctactgatgt	tcattccagaa	gatattccct	acttgaaaaa	ggcttccgat	300
ggttttacaac	cagaagttac	tagattcttg	gaacaacatt	ccccagattg	gatcatctac	360
gattatactc	attactgggt	gccattccatt	gctgcttcat	tgggtatttc	tagagcccat	420
ttctctgtta	ctactccatg	ggctattgct	tatatgggtc	catctgctga	tgctatgatt	480
aacggttctg	atggtagaac	taccgttgaa	gatttgacta	ctccaccaa	gtggtttcca	540
tttccaacaa	aagtctgttg	gagaaaacac	gatttggtta	gattgggtcc	atacaaagct	600
ccaggatttt	ctgatgggtta	cagaatgggt	atgggtttga	aagggtccga	ttgcttggtg	660
tctaagtgtc	atcatgaatt	cggtactcaa	tgggtgcctt	tggttgaaac	attgcatcaa	720
gttccagttg	ttccagtagg	ttgttgcca	ccagaaattc	cagggtgacg	aaaagacgaa	780
acttgggttt	ccatcaaaaa	gtggttggat	ggtaagcaaa	agggttctgt	tgtttatgtt	840
gctttgggtt	ccgaagcttt	ggtttctcaa	accgaagttg	ttgaattggc	tttgggtttg	900
gaattgtctg	gtttgccatt	tgtttgggtc	tacagaaaac	ctaaagggtc	agctaagtct	960
gattctgttg	aattgccaga	tggtttcgtt	gaaagaacta	gagatagagg	tttggtttgg	1020
acttcttggg	ctccacaatt	gagaattttg	tctcatgaat	ccgtctgttg	tttcttgact	1080
cattgtgggt	ctgggttctat	cgttgaagg	ttgatgtttg	gtcaccctat	gattatgttg	1140
ccaatctttg	gtgaccaacc	attgaacgct	agattattgg	aagataagca	agtcgggtatc	1200
gaaatcccaa	gaaatgaaga	agatgggtgc	ttgaccaaag	aatctgttgc	tagatctttg	1260
agatccgttg	tcgttgaaaa	agaagggtga	atctacaagg	ctaacgctag	agaattgtcc	1320
aagatctaca	acgataccaa	ggtcgaaaaa	gaatacgttt	cccaattcgt	tgactacttg	1380
gaaaagaatg	ctagagctgt	tgccattgat	catgaatctt	ga		1422

SEQ ID NO:12

Oryza sativa sequence encoding EUGT11 (codon optimized)

atggatagtg	gctactcctc	atcttatgct	gctgccgctg	gtatgcacgt	tgtgatctgc	60
ccttggttgg	cctttgggtc	cctgttacca	tgtctggatt	tagcccaaag	actggcctca	120
agaggccata	gagtatcatt	tgtgtctact	cctagaaata	tctctcgttt	accaccagtc	180
agacctgctc	tagctcctct	agttgcattc	gttgctcttc	cacttccaag	agtagaagga	240
ttgccagacg	gcgctgaatc	tactaatgac	gtaccacatg	atagacctga	catggtcgaa	300
ttgcatagaa	gagcctttga	tggattggca	gctccatttt	ctgagttcct	gggcacagca	360
tgtgcagact	gggttatagt	cgatgtatct	catcactggg	ctgctgcagc	cgcatgggaa	420
cataagggtg	cttgtgctat	gatgttggtt	gggtcagcac	acatgatcgc	atccatagct	480
gatagaagat	tggaaagagc	tgaacagaa	tccccagccg	cagcaggaca	aggtaggcca	540
gctgccgccc	caacctttga	agtggctaga	atgaaattga	ttcgtactaa	aggtagttca	600
gggatgagtc	ttgctgaaag	gttttctctg	acattatcta	gatcatcatt	agttgtaggt	660
agatcctgcg	tcgagttcga	acctgaaaca	gtacctttac	tatctacttt	gagaggcaaa	720
cctattactt	tccttgggtc	aatgcctcca	ttacatgaag	gaaggagaga	agatgggtgaa	780
gatgctactg	ttaggtgggt	agatgcccac	cctgctaagt	ctgttggtta	cgttgcattg	840
ggttctgagg	taccactagg	ggtggaaaag	gtgcatgaat	tagcattagg	acttgagctg	900
gccggaacaa	gattcctttg	ggctttgaga	aaaccaaccg	gtgtttctga	cgccgacttg	960
ctaccagctg	ggttcgaaga	gagaacaaga	ggccgtgggt	tcgttgctac	tagatgggtc	1020
ccacaaatga	gtattctagc	tcattgcagc	gtaggggcct	ttctaaccac	ttgcgggttg	1080

```

aactcaacaa tagaaggact gatgtttggt catccactta ttatgttacc aatctttggc      1140
gatcagggac ctaacgcaag attgattgag gcaaagaacg caggtctgca gggtgcacgt      1200
aatgatgggtg atggttcctt tgatagagaa ggcgttgacg ctgccatcag agcagtcgcc      1260
gttgaggaag agtcatctaa agttttccaa gctaaggcca aaaaattaca agagattgtg      1320
gctgacatgg cttgtcacga aagatacatc gatggtttca tccaacaatt gagaagttat      1380
aaagactaa                                1389

```

SEQ ID NO:13

YBR180W

```

>sp|P38125|DTR1_YEAST Dityrosine transporter 1 OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=DTR1 PE=1 SV=1
MGSEPFQKKNLGLQINSQESGTTTRSTFHSLEDLGDDVINESWDQVNQKRANIDHDFHEH
PDSSPSLSAQKAKTKEEEVAVKSSNSQSRDSPDTQAHIPYTYFSKDQRLIIFGIIIFIG
FLGPMMSGNIYIPALPLLQREYDVSATTINATVSVFMAVFSVGPLFWGALADFGGRKFLYM
VSLSLMLIVNILLAAVPVNIAALFVLRI FQAFASSSVISLGAGTVTDVVPPKHRGKAIAY
FMMGPNMGPIIAPIVAGLILMKGN YWRWLF GFTSIMTGIALILVTALLPETLRCIVGNGD
PKWGDKKDERENNESPFEGNKISHRRLFPDIGIRKPVNND AFFQENFPKPPKAGLTLYW
KMIKCPPIIITSVSTALLFSSYYAFSVTF SYYLEHDYRFTMLEIGAAYVCPGVAMLLGSQ
SGGHLSDYLRSRWIKSHPKKKFPAEFRLLLNLIGILLTICGTIGYGWAIFFHYHFVLLV
FSALTAFGMTWCSNTSMTYLTELF PKRAAGTVAVSSFFRNVGAAISSAIILQLCNAMGIG
WCFTGLGLCSSISLIGILYLLIFQRKYTAKEF

```

SEQ ID NO:14

YAL067C

```

>sp|P39709|SEO1_YEAST Probable transporter SEO1 OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=SEO1 PE=1 SV=1
MYSIVKEIIVDPYKRLKWGFIPVKRQVEDLPDDLNSTEIVTISNSIQSHETAENFITTS
EKDQLHFETSSYSEHKDNVNVTRSYEYRDEADRPWWRFFDEQEYRINEKERSHKNWYSWF
KQGTSFKEKKLLIKLDVLLAFYSCIA YWVKYLD TVNINNAYVSGMKEDLGFQGNDLVHTQ
VMYTVGNIIIFQLPFLIYLNKLPLNYVLPSLDLCWSLLTVGAAYVNSVPHLKAIRFFIGAF
EAPSYLAYQYLFGSFYKHDEMVRRSAFY YLGQYIGILSAGGIQSAVYSSLNGVNGLEGWR
WNFIIDAIVSVVVG LIGFYSLPGDPYNCYSIFLTDDEIRLARKRLKENQTGKSDFETKVF
DIKLWKTIFSDWKIYILTLWNI FCWNDSNVSSGAYLLWLKSLKRY SIPKLNQLSMITPGL
GMVYLMLTGIIADKLHSRWF AII FTQVFNI IGNSILA AWDVAEGAKWFAFMLQCFGWAMA
PVLYSWQNDICRRDAQTRAITLV TMNIMAQSSTAWISVLVWKTEEAPRYLKGFTFTACSA
FCLSIWTFVVLYFYKRDERNNAKKNGIVLYNSKHGVEKPTSKDVETLSVSDEK

```

SEQ ID NO:15

YBL089W

```

>sp|P38176|AVT5_YEAST Vacuolar amino acid transporter 5
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=AVT5
PE=3 SV=2
MPSNVRSGVLTLLHTACGAGVLAMPFAFKPFGLMPGLITLTF CGICSLCGLLLQTRIAKY
VPKSENASF AKLTQLINPSISVVFDFAIAVKCFGVGVSYLIIVGDLVPQIVQSIFYRND
NMSGSQEHMFLDRRLYITLIIVFVISPLCFKRSLNSLRYASMI AIVSVAYLSGLIIYHF
VNRHQLERGVYFMVPHGDSQSHSPLTTLPIFVFAYTCHNMFSVINEQVDKSFKVIRRI
PIFAIVLAYFLYIIIGGTGYMTFGENIVGNILTLYPNSISTTIGRLAMLLLVMLAFPLQC
HPCRSSVKNIIIFIENFRKGKLYDNRASFIPLDNFNSEDPQEAPTQQNNEEPNLRSESLR

```

HINIITLCILLFSYLLAISITSLAKVLAIVGATGSTSISFILPGLFGYKLGISEFTGTNE
RVPTSIKIFKYLSLSLFIWGIAVMVASLSAIVFLGTSSH

SEQ ID NO:16

YBL099W

>sp|P07251|ATPA_YEAST ATP synthase subunit alpha, mitochondrial
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ATP1
PE=1 SV=5

MLARTAAIRSLRSTLINSTKAARPAALASTRRLASTKAQPTDEVSSILEERIKGVSD
EALNLNETGRVLAVGDGIARVFGGLNNIQAEELVEFSSGVKGMALNLEPGQVGIVLFGSDRLVK
EGELVKRTGNIVDVPVGPGLLGRVVDALGNPIDGKGPIAAGRSRAQVKAPGILPRRSVH
EPVQTGLKAVDALVPIGRGQRELIIGDRQTGKTAVALDTILNQKRWNNGSDESKKLYCVY
VAVGQKRSTVAQLVQTLQHDAMKYSIIVAATASEAAPLQYLAPFTAASIGEWFRDNGKH
ALIVYDDLKQAVAYRQLSLLLRPPGREAYPGDVLYLHSRLLERAALKEKEGSGSLTA
LPVIETQGGDVSAIIPITNVISITDGQIFLEAEFYKGRPAINVGLSVSRVGSAAQVKAL
KQVAGSLKLFLAQYREVAFAAQFGSDLDASTKQTLVRGERLTQLLKQYQYSPLATEEQVP
LIYAGVNGHLDGIELSRIGEFESSFLSYLKSNNHLLTEIREKGELSKELLASLKSATES
FVATF

SEQ ID NO:17

YBR241C

>sp|P38142|YB91_YEAST Probable metabolite transport protein YBR241C
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YBR241C
PE=1 SV=1

MAETERLMPNGGSRETKPLITGHLILGTIVACLSIQYGYHIAELNAPQEFLLSCSRFEAP
DENISYDDTWVGQHGLKQCIALTDSQYGAITSIFSIGGLFGSYYAGNWANRYGRKYVSMG
ASAMCMVSSLLFFSNSYLQLLFGRFLVGMSCGTAVITPLFINEIAPVEWRGAMGSMNQ
VSINLGILLTQTLALKYADSYNWRWLLFSGSVIAVANILAWLKVDESPRWLVSHGFVSEA
ETALFKLRPGTYQQAKQEIQDWQRSHGHNRDPESSEETHSGPTLWQYVTDPSYKKPRTVI
LAILSCQQFCGINSIIFYGVKVGKILPDYSIQVNFAISILNVVVTLAASAIIDHVGRRP
LLLASTTVMTAMSLISVGLTSLSVSFLLVATFVYIAAFAIGLGPFLIIGELSYQPDA
ATAQSFGTVCNWLATFIVGYLFPIGHGLMGGYVFAIFAAIAAMFATYVYKRVPETKGKTT
YSEVWAGY

SEQ ID NO:18

YBR294W

>sp|P38359|SUL1_YEAST Sulfate permease 1 OS=Saccharomyces cerevisiae
(strain ATCC 204508 / S288c) GN=SUL1 PE=1 SV=2

MSRKSSTEYVHNQEDADIEVFESYRITYRESEAAENRDGLHNGDEENWKVNSSKQKFGVT
KNELSDVLYDSIPAYEESTVTLKEYYDHSIKNNLTAKSAGSYLVSLFPPIKWFPHYNFTW
GYADLVAGITVGCVLVPQSMSYAQIASLSPEYGLYSSFIGAFIYSLFATSKDVCIGPVAV
MSLQTAKVIAEVLKKYPEDQTEVTAPIIATTLCLLCGIVATGLGILRLGFLVELISLNAV
AGFMTGSAFNI IWGQIPALMGYNLSLVNTREATYKVVINTLKHLPNTKLDVFGLIPLVIL
YVWKWWCGTFGITLADRYRNPQPKVANRLKSFYFYAQAMRNAVVIVVFTAISWSITRNS
SKDRPISILGTVPISGLNEVGVMKIPDGLLSNMSSEIPASIIVLVLEHIAISKSFGGRINDY
KVVPDQELIAIGVTNLIGTFFHSYPATGSFSRSALKAKCNVRTPFSGVFTGGCVLLALYC
LTDAFFFIPKATLSAVIIHAVSDLLTSYKTTWTFWKTNPDCISFIVTVFITVFSSIENG
IYFAMCWSCAMLLKQAFPAGKFLGRVEVAEVLNPTVQEDIDAVISSNELPNELNKQVKS
TVEVLPAPPEYKFSVKWVPFDHGYSRELNINTTVRPPPPGVIVYRLGDSFTYVNCSSRHYDI
IFDRIKEETRGRQLITLRKKSDRPWNPDGEWKMPDSLKSLFKFKRHSATTNSDLPISNGS
SNGETYEKPLLKVVCLDFSQVAQVDSTAVQSLVDLRKAVNRYADRQVEFHFIISPWIK

RSLLSVKFGTTNEEYSDDSI IAGHSSFHVAKVLKDDVDYTD EDSRISTSYSNYETLCAAT
GTNLPPFFHIDI PDFSKWDV

SEQ ID NO:19

YCL069W

>sp|P25594|VBA3_YEAST Vacuolar basic amino acid transporter 3
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=VBA3
PE=1 SV=1

MNMLIVGRVVASVGGSGLQTL CFVIGCTMVGERSRPLVISILSCAFAVA AIVGPIIGGAF
TTHVTWRWCFYINLP IGG LAIIMFLLTYKAENKGILQQIKDAIGT ISSFTFSKFRHQVNF
KRLMNGIIFKFDFFGFALCSAGLV LFLGLTFGGNKYSWNSGQVIAYLV LGVLLFIFSLV
YDFFLFDKFNPEPDNISYRPLLRLVAKPAII IINMVTFL LCTGYNGQMIYSVQFFQLI
FASSAWKAGLHLIPIVITNVIAA IASGVITKKLGLVKPLLI FGGVLGVIGAGLMTLMTNT
STKSTQIGVLLLPGFSLGFALQASLMSAQLQITKDRPEAAMDFIEVTA FNTFMKSLGTTL
GGVLSTTVFSASFHNKVSRAHLEPYEGKTVDDMILYRLQNYDGSHSTIGNILSDSIKNVF
WMDLGFYALGFLFCSFSSNKKLIIPKKDETPEDNLEDK

SEQ ID NO:20

YCR028C

>sp|P25621|FEN2_YEAST Pantothenate transporter FEN2 OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=FEN2 PE=1 SV=1

MMKESKSITQHEVERESVSSKRAIKRLLL FKIDLFVLSFVCLQYWINYVDRVGFTNAYI
SGMKEDLKMVGNDLTVSNTVFMIGYIVGMVPNNLMLLCVPPRIWLSFCTFAWGLLTGMY
KVTSFKHICAIRFFQALFESCTFSGTHFVLGSWYKEDELPIRSAIFTGSGLVGSMFSGFM
QTSIFTHLNGRNLGAGWRWLFII DFCITLPIAIYGFIFFPGLPDQTS AVSKFSMTRYIFN
EQELHYARRRLPARDESTRLDWSTIPRVLKRWHWWMFSLVWVLGGENLGFASNSTFALWL
QNQKYTLAQRNNYP SGIFAVGIVSTLCSAVYMSKI PRARHWHVSVFISLVMVIVAVLIRA
DPLNPKVVFS AQYLGGVAYAGQAVFFSWANI ICHADLQERAIVLASMNMFSGAVNAWWSI
LFFASDMVPKFERG CYALLATAISSGIVSVVIRSLQIKENLSKKQVPYIDANDMPGEDDD
DDNQDNENDGDDESMEVELHNEEMAEISNPFR

SEQ ID NO:21

YCR075C

>sp|P17261|ERS1_YEAST Cystine transporter OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=ERS1 PE=1 SV=1

MVSLDDILGIVYVTSWSISMYPPIITNWRHKSASAISMDFVMLNTAGYSYLVISIFLQLY
CWKMTGDESDLGRPKLTQFDFWYCLHGCLMNVLLTQVVAGARIWRFPKGKGRKMNPWYL
RILLASLAIFSLLT VQFMYSNYWYDWHNSRTLAYCNNL FLLKISMSLIKYPQVTHNSTR
KSMDCFP IQGVFLDVTGGIASLLQLIWQLSNDQGFSLDTFVTNFGKVGLSMVTLI FNFIF
IMQWFVYRSRGHDLASEYPL

SEQ ID NO:22

YDL128W

>sp|Q99385|VCX1_YEAST Vacuolar calcium ion transporter
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=VCX1
PE=1 SV=1

MDATTPLLTVANSHPARNP KHTAWRAAVYDLQYILKASPLNFLLVFVPLGLIWHGFQLSH
TLTFLFNFLAI IPLAAILANATEELADKAGNTIGLLNATFGNAVELIVSIIALKKGQVR
IVQASMLGSLLSNLLLVLGLCFIFGGYNRVQQTFNQTA AQTMSSLLAIACASLLIPAAFR

ATLPHGKEDHFIDGKILELSRGTSIVILIVYVLFYFQLGSHHALFEQQEEETDEVMSTI
 SRNPHSLSVKSSLVILLGTTVIIISFCADFLVGTIDNVVESTGLSKTFIGLIVIPIVGNA
 AEHVTSVLVAMKDKMDLALGVAIGSSLQVALFVTFPMVLVGMIDVPMTLNFSTFETATL
 FIAVFLSNYLILDGESNWLEGVMSLAMYILIAMAFFYYPDEKTLDSIGNSL

SEQ ID NO:23

YDL185W

>sp|P17255|VATA_YEAST V-type proton ATPase catalytic subunit A
 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=VMA1
 PE=1 SV=3

MAGAIENARKEIKRISLEDHAESEYGAIYSVSGPVVIAENMIGCAMYELVKVGHNDNLVGE
 VIRIDGDKATIQVYEETAGLTVGDPVLRGTGKPLSVELGPGLMETIYDGIQRPLKAIKEES
 QSIYIPRGIDTPALDRTIKWQFTPGKFQVGDHISGGDIYGSVFENSLISSHKILLPPRSR
 GTITWIAPAGEYTLDEKILEVEFDGKKSDFTLYHTWPVRVPRPVTEKLSADYPLLTGQRV
 LDALFPCVQGGTTCIPGAFGCGKTVISQSLSKYSNSDAIIYVGCFAGKGTNVLMADGSIEC
 IENIEVGKVMGKDGRPREVIKLPGRGRETMYSVVQKSQHRAHKSDDSSREVPPELLKFTCNA
 THELVVRTPRSVRRLSRTIKGVEYFEVITFEMGQKKAPDGRIVELVKEVSKSYPISEGPE
 RANELVESYRKASNKAYFEWTIEARDLSLLGSHVRKATYQTYAPILYENDHFFDYMOKSK
 FHLTIEGPKVLAYLLGLWIGDGLSDRATFSVDSRDTSLMERVTEYAEKLNLCAYKDRKE
 PQVAKTVNLYSKVVRGNGIRNNLNTENPLWDAIVGLGFLKDGVKNI PSFLSTDNI GTRET
 FLAGLIDSDGYVTDEHGIKATI KTIHTSVRDGLVSLARSLGLVSVNAEPAKVDMNGTKH
 KISYAIYMSGGDVLLNVLSKCAGSKKFRPAPAAFARECRGFYFELQELKEDDYGITLS
 DDSDHQFLLANQVVVHNCGERGNEMAEVLMEFPELYTEMSGTKEPIMKRTTLVANTSNMP
 VAAREASIYTGITLAEYFRDQGKNVSMIADSSSRWAEALREISGRLGEMPADQGFPAYLG
 AKLASFYERAGKAVALGSPDRTGSVSIVA AVSPAGGDFSDPVT TATLGITQVFWGLDKKL
 AQRKHFPSINTSVSYSKYTNVLNKFYDSNYPEFPVLRDRMKEILSNAEELEQVVQLVGKS
 ALSDSKITLDVATLIKEDFLQQNGYSTYDAFCPIWKTFDMMRAFISYHDEAQKAVANGA
 NWSKLADSTGDVKHAVSSSKFFEPSRGEKEVHGEFEKLLSTMQERFAESTD

SEQ ID NO:24

YDL194W

>sp|P10870|SNF3_YEAST High-affinity glucose transporter SNF3
 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SNF3
 PE=1 SV=3

MDPNSNSSSETLRQEKQGF LDKALQRVKGIALRRNNSNKDHTTDDTTGSIRTPTSLQRQN
 SDRQSNMTSVFTDDISTIDDNSILFSEPPQKQSMMSICVGVFVAVGGFLFGYDTGLINS
 ITSMNYVKSHVAPNHDSFTAQQMSILVSFLSLGTFFGALTAPFISDSYGRKPTIIFSTIF
 IFSIGNSLQVGAGGITLLIVGRVISGIGIGAISAVVPLYQAEATHKSLRGAIISTYQWAI
 TWGLLVSSAVSQGTHARN DASSYRIPIGLQYVWSSFLAIGMFFLPESPRYYVLKDKLDEA
 AKSLSFLRGVPVHDSGLLEELVEIKATYDYEASFGSSNFIDCFISSKSRPKQTLRMFTGI
 ALQAFQQFSGINFIFYYGVNFFNKTGVSNSYLVSFITYAVNVVFNVPGLFFVEFFGRRKV
 LVVGGVIMTIANFIVAIVGCSLKTVA AAKVMIAFICLFIAAFSATWGGVWVVISAE LYPL
 GVRSKCTAICAAANWLVNFICALITPYIVDTGSHTSSLGAKIFFIWGSLNAMGVIVVYLT
 VYETKGLTLEEIDELYIKSSTGVVSPKFNKDIRERALKFQYDPLQRLEDGKNTFVAKRNN
 FDDETPRNDFRNTISGEIDHSPNQKEVHSIPERVDIPTSTEILESPNKSSGMTVPVSPSL
 QDVPIPQTTEPAEIRTKYVDLGNGLGLNTYNRGPPSLSSDSSSEDYTEDEIGGPSSQGDQS
 NRSTMNDINDYMARLIHSTSTASNTTDKFSGNQSTLRYHTASSHSDTTEEDSNLMDLGNG
 LALNAYNRGPPSILMNSSDEEANGGETSDNLNTAQDLAGMKERMAQFAQSYIDKRGGLEP
 ETQSNILSTSL SVMADTNEHNNEILHSSEENATNQPVNENNDLK

SEQ ID NO:25

YDL210W

>sp|P32837|UGA4_YEAST GABA-specific permease OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=UGA4 PE=1 SV=1
 MSMSSKNENKISVEQRISTDIGQAYQLQGLGSNLSIRSKTGAGEVNYIDAAKSVNDNQL
 LAEIGYKQELKRQFSTLQVFGIAFSIMGLLPSIASVMGGGLGGGPATLVWGWFFVAAFFIL
 LVGITMAEHASSIPTAGGLYYWTYYYAPEGYKEIISFIIGCSNSLALAAGVCSIDYGLAE
 EIAAAVTLTKDGNFEVTSGKLYGIFAGAVVVMCICTCVASGAIARLQTLISIFANLFIIIVL
 LFIALPIGTKHRMGGFNDGDFIFGKYENLSDWNNGWQFCLAGFMPAVWTIGSFDSCVHQS
 EEAKDAKKSVPIGIISSIAVCWILGWLIIICLMACINPDIDSVLDSKYGFALAQIIYDSL
 GKKWAI AFMSLIAFCQFLMGASITTAVSRQVWAFSRDNGLPLSKYIKRVDSKYSVPFFAI
 LAACVGSLLILGLLCLIDDAATDALFSLAVAGNNLAWSTPTVFRLTSGRDLFRPGPFYLGK
 IWSPIVAWTGVAFQLFIIILVMFPSQQHGITKSTMNYACVIGPGIWILAGIYYKVYKKKY
 YHGPATNLSDDDYTEAVGADVIDTIMSKQEP

SEQ ID NO:26

YDR061W

>sp|Q12298|YD061_YEAST Uncharacterized ABC transporter ATP-binding protein YDR061W OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YDR061W PE=1 SV=1
 MSTNKFVVRITNALFKSSLASNSPPVYPKRIRHFEILPNEKWVIWGPKGKFLDVLNNKY
 ICEPPLSLRFGFLKESSNILPRIEQVAFKGVMPHTAHLARSARYEYFKDDYDQTCQFIFDKA
 SGSNAVSYSKQVETNNRQINMELYNALVENLNLSSLQDRWVMGLSNGQMRRARLARSILKEP
 DLLLIDDPFLGLDPAAIATISQFLAKYDSIEVSGGCPIVIGLRYQDTIPAWCTHICCVDE
 KNGILFEGPIEKLQSKMDETRSRALKELEQLKKASNSKEDISINDLICIHPMYGKKEHEI
 IKMPHLIELDGLSVSYKGEAVLENLHWKVQPGSKWHIRGDNGSGKSTLLSLLTAEHPQSW
 NSRVIDNGVPRRTGKTNYFDLNSKIGMSSPELHAI FLKNAGGRLNIRESVATGYHEASSN
 NYLPIWKRLDKNSQEIVNMYLKYFGLDKDADSVLFEQLSVSDQKLVLVFRSLIKMPQILI
 LDEAFSGMEVEPMMRCHEFLEEWPGTVLVVAHVAEETPKCAHYLRLISPGEYEIGDMEN

SEQ ID NO:27

YDR093W

>sp|Q12675|ATC4_YEAST Phospholipid-transporting ATPase DNF2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=DNF2 PE=1 SV=1
 MSSPSKPTSPFVDDIEHESGSASNGLSSMSPFDDSFQFEKPSSAHGNIEVAKTGGSVLKR
 QSKPMKDISTPDLSKVTFDGDIDYSDNDNDINDDELNGKKTEIHEHENEVDDDLHSFQAT
 PMPNTGGFEDVELDNNEGSNNDSQADHKLKRVRFGTRRNKSGRIDINRSKTLKWAKKNFH
 NAIDDEFSTKEDSLENSALQNRSEDLRTVYYNLPLPEDMLDEDGLPLAVYPRNKIRTTKYT
 PLTFFPKNILFQFHNFANIYFLILLILGAFQIFGVTNPGFASVPLIVIVIITAIKDGIED
 SRRTVLDLEVNNTTRTHILSGVKNENVAVDNVSLWRRFKKANTRALIKIFEYFSENLTAAAG
 REKKLQKKREELRRKRNSRSFGPRGSLDSIGSYRMSADFGPSLDYENLNQTMSQANRYN
 DGENLVDRTLQPNPECFADYWKNVKVGDIVRVHNNDEIPADMILLSTSDVDGACYVET
 KNLDGETNLKVRQSLKCSKIIKSSRDITRTKFWVESEGPANLYSYQGNFKWQDTQNGNI
 RNEPVNINNNLLRGCTLRNTKWAMGMVIFTGDDTKIMINAGVTPTKKSRI SRELNFSVIL
 NFVLLFILCFTAGIVNGVYYKQKPRSRDYFEFGTIGGSASTNGFVSFWVAVILYQSLVPI
 SLYISVEIIKTAQAIFIYTDVLLYNAKLDYPCTPKSWNISDDLQIEYIFSDKTGTLTQN
 VMEFFKCTINGVSYGRAYTEALAGLRKRQGV DVESEGRREKEEIAKDRETMIDELRMSD
 NTQFCPEDLTFVSKEIVEDLKGSSGDHQQKCCEHFLLALALCHSVLVEPNKDDPKKLDIK
 AQSPDESALVSTARQLGYSFVGSSKSGLIVEIQGVQKEFQVLNVLEFNSSSRKRMSCIKI
 PGSTPKDEPKALLICKGADSVIYSRLDRTQNDATLLEKTALHLEEYATEGLRTLCLAQRE
 LTWSEYERWVKTYDVAAASVTNREEELDKVTDVIERELILLGGTAIEDRLQDGV PDSIAL

LAEAGIKLWVLTGDKVETAINIGFSCNVLNNDMELLVVKASGEDVEEFGSDPIQVVNNLV
 TKYLREKFGMSGSEELKEAKREHGLPQGNFAVIIDGDALKVALNGEEMRRKFLLLCKNC
 KAVLCCRVSPAQKAAVVKLVKKTLDVMTLAIGDGSNDVAMIQSADVGVGIAGEEGRQAVM
 CSDYAIGQFRYVTRLVLVHGKWCYKRLAEMIPQFFYKNVIFTLSLFWYGIYNNFDGSYLF
 EYTYLTFYNLAFTSVPVILLAVLDQDVSDTVSMLVPQLYRVGILRKEWNQTKFLWYMLDG
 VYQSVICFFFPYLAYHKNMVVTENGLGLDHRYFVGVFVTAIAVTSCNFYVFMEQYRWDWF
 CGLFICLSLAVFYGWTGIWTSSSSSNEFYKGAARVFAQPAYWAVLFVGVLFCLLPRFTID
 CIRKIFYPKDIEIVREMWLRGDFDLYPQGYDPTDPSRPRINEIRPLTDFKEPISLDTHFD
 GVSHSQETIVTEEIPMSILNGEQGSRKGYRVSTTLERRDQLSPVTTTNNLPRRSMASARG
 NKLRTSLDRTREEMLANHQLDTRYVERARASLDLPGINHAETLLSQRSRDR

SEQ ID NO:28

YDR338C

>sp|Q05497|YD338_YEAST Uncharacterized transporter YDR338C
 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YDR338C
 PE=1 SV=1

MAGILSKTLSEVHPSLRTNGMGIGNTHRRISLGFLPPNKKNPLVRKFRARTRNIDQRSFR
 SLTDDFGSNVHEPNPYLGNIDEEDPDLYYHDEEDGELSRTISLPSRVSETPELSPQDVDWI
 LHEHERRYSSVCNSDNEEASQSNTPDRIQEYSGRELEYDEFMNRLQAQKQKLTRSAVTDA
 KGTSHHRRPSFVSVTSRGSVPTIYQEIDENDSEALAEASHVTFKSEARVLASYSFPLI
 FTFLEQIFPMVCSLTVGHLGKNELAAVSLASMTSNITLAI FEGIATSLDTLCPQAYGSG
 RFYSVGVHLQRCIAFSLVIYIPFAVMWWYSEPLLSYIIPEKELINLTSRFLRVLILGAPA
 YIFFENLKRFLQAQGI FDAGIYVLTICAPLNVLVSYTLVWNKYIGVGFIGAAIAVVLNFW
 LMFFLLLFYALYIDGRKCWGGFSRKAFTHWNDLGHLAFSGIIMLEAEELSYELLTLFSAY
 YGVSYLAAQSAVSTMAALLYMIPFAIGISTSTRIANFIGAKRTDFAHISSQVGLSFSFIA
 GFINCCILVFGRNLIANIYSKDPEVIKLIQVLPLVGIVQNFDSLNAVAGSCLRGQGMQS
 LGSIVNLMAYYLFGIPLALILSWFFDMKLYGLWIGIGSAMLIGLVEAYYVLPDWDKIM
 TYAEILKETEDDEVDSDEYLTDSDDPDENTALLGA

SEQ ID NO:29

YDR406W

>sp|Q04182|PDR15_YEAST ATP-dependent permease PDR15 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=PDR15 PE=1 SV=1

MSSDIRDVEERNRSRSSSSSSSSNSAAQSIGQHPYRGFDSEAAERVHELARTLTSQSLLYT
 ANSNSSSSSNHNAHNADSRVSFSTDMEGVNPVFTNPDTPGYNPKLDPNSDQFSSTAWVQN
 MANICTSDPDFYKPYSLGCVWKNLSASGDSADVSQSTFANIVPKLLTKGLRLLKPSKEE
 DTFQILKPMDGCLNPGELLVVLGRPGSGCTTLLKSISNSHGFKIAKDSIVSYNGLSSSD
 IRKHRYGEVVYNAESDIHLPHLTVYQTLFTVARMKTPQNRIKGVDRAYANHVTEVAMAT
 YGLSHTRDTKVGNDLVRGVSGGERKRVSAEVAICGARFQCWDNATRGLDSATALEFIRA
 LKTQADIGKTAATVAIYQCSQDAYDLFDKVCVLDDGYQLYFGPAKDAKKYFQDMGYYP
 RQTTADFLTSITSPTERIISKEFIEKGTRVPQTPKDMAEYWLQSESYKNLIKDIDSTLEK
 NTDEARNIIRDAHAKQAKRAPPSSPYVVNYGMQVKYLLIRNFWRMKQSASVTLWQVIGN
 SVMAFILGSMFYKVMKKNDTSTFYFRGAAMFFAILFNAFSCLEIFSLYETRPITEKHRT
 YSLYHPSADAFASVLSEMPKLTAVCFNIIFYFLVDFRRNGGVFFFYFLINVIATFTLS
 HLFRCVGS�TKTLQEAMVPASMLLLAISMYTGFAIPKTKILGWSIWIWYINPLAYLFESL
 MINEFHDRRFPCAQYIPAGPAYQNITGTQRVCSAVGAYPGNDYVLGDDFLKESYDYEHHK
 KWRGFGIGMAYVVFFFFVYLILCEYNEGAKQKGEMVVFLRSKIKQLKKEGKLQEKHRPGD
 IENNAGSSPD SATTEKKILDDSSSEGS DSSSDNAGLGLSKSEAI FHWRDLCYDVPIKGGQR
 RILNNVDGWVKPGTTLTALMGASGAGKTTLLDCLAERVMTMGVITGNI FVDGRLRDESFPRS
 IGYCQQQDLHLKTATVRESLRFSAYLRQPSSVSIEEKNRYVEEVIKILEMQQYSDAVVGV
 AGEGLNVEQRKRLTIGVELAARPKLLVFLDEPTSGLDSQTAWDTCQLMRKLATHGQAILC
 TIHQPSAILMQQFDRLFLQKGGQTVYFGDLGEGCKTMIDYFESKGAHKCPPDANPAEWM

LEVVGAAAPGSHATQDYNEVWRNSDEYKAVQEELDWMEKNLPGRSKEPTAEHHPFAASLY
YQFKMVTIRLFQQYWRS PDYLWSKFILTI FNQVFIGFTFFKADRSLOGLQNQMLSI FMYT
VIFNPILQQYLPSFVQQORDLYEARERPSRTFSWLAFFLSQIIVEIPWNILAGTIA YCIYY
YAVGFYANASAAGQLHERGALFWLFSIAFYVYIGSMGLLMISFNEVAETA AHMGTL LFTM
ALSFCGVMATPKVMPRFWIFMYRV SPLTYMIDALLALGVANVDVKCSNYEMVKFTPPSGT
TCGDYMASYIKLAGTG YLS DPSATDICS FCAVSTTNAFLATFSSHYYRRWRNYGIFICYI
AFDYIAATFLYWLSRV PKKNGKISEKPKK

SEQ ID NO:30

YDR536W

>sp|P39932|STL1_YEAST Sugar transporter STL1 OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=STL1 PE=1 SV=2
MKDLKLSNFKGKFISRTSHWGLTGKKLRYFITIASMTGFSLFGYDQGLMASLITGKQFNY
EFPATKENG DHD RHATVVQGATTSCYELGCFAGSLFVMFCGERIGRKPLILMGSVITIIG
AVISTCAFRGYWALGQFIIGRVVTGVGTGLNTSTIPVWQSEMSKAENRGLLVNLEGSTIA
FGTMIAYWIDFGLSYTNSSVQWRFPVSMQIVFALFLLAFMIKLPESPRWLISQSRTEEAR
YLVGTLDDADPNDEEVITEVAMLHDAVNRTKHEKHSLSLFSRGRSQNLQORALIAASTQF
FQQFTGCNAAIYYSTVLFNKTIKLDYRLSMIIGGVFATIIYALSTIGSFFLIEKLGRKRLF
LLGATGQAVSFTITFACLVKENKENARGAAVGLFLFITFFGLSLLSLPWIYPPEIASMKV
RASTNAFSTCTNWLCNFAVVMFTPIFIGQSGWGCYLF FAVMNYLYIPVIFFFYPETAGRS
LEEIDII FAKAYEDGTQ PWRVANHLPKLSLQEVEDHANALGSYDDEMEKEDFGEDRVEDT
YNQINGDNSSSSSSNIKNEDTVNDKANFEG

SEQ ID NO:31

YEL031W

>sp|P39986|ATC6_YEAST Manganese-transporting ATPase 1
OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SPF1
PE=1 SV=1
MTKKS FVSSPIVRDSTLLVPKSLIAKPYVLPFFPLYATFAQLYFQQYDRYIKGPEWTFVY
LGTLVSLNILVMLMPAWN VKIKAKFNYSTTKNVNEATHILIIYTT PNNGSDGIVEIQRVTE
AGSLQ TFFQFQKKRFLWHENEQVFSSPKFLVDESPKIGDFQKCKGHSGDLTHLKRLYGEN
SFDIPIPTFMELFKEHAVAPLFVFQVFCVALWLLDEFWYYSLFNLFMIISMEAAAVFQRL
TALKEFRTMGIKPYTINVERNKKWVALQTNELLPMDLVSITRTAEESAIPCDLILLDGS
IVNEAMLSGESTPLLKESIKLRPSEDNLQLDGVDKIAVLHGGTKALQVTPPEHKSDIPPP
PDGGALAI VTKTG FETSQGS LVRVMIYSAERVSVDNKEALMFILFLLIFAVIASWYVWVE
GTKMGRIQSKLILDCILIIITSVVPPELPMELTMAVNSSLAALAKFYVYCTEPFRI PFAGR
IDVCCFDKTGTTLTGEDLVFEGLAGISADSENIRHLYSAAEAPESTILVIGAAHALVKLED
GDIVGDPMEKATLKAVGWAVERKNSNYREGTGKLDIIRRFQFSSALKRSASIASHNDALF
AAVKGAPETIRERLS DIPKNYDEIYKS FTRSGSRVLALASKSLPKMSQSKIDDLNRDDVE
SELT FNGFLIFHCPLKDDAIETIKMLNESSHRSIMITGDNPLTAVHVAKEVGIVFGETLI
LDRAGKSDDNQLLFRDVEETVSI PFDPSKDTFDH SKLFD RYDIAVTGYALNALEGHSQLR
DLLRHTWVYARVSPSQKEFLLNTLKD MG YQ TLMCGDGTNDVGALKQAHVGIAL LINGTEEG
LKKLGEQRRLEGMKMMYIKQTEFMARWNQPQPPVPEPIAHLFP PGPKNPHYLKALESKGT
VITPEIRKAVEEANSKPVEVIKPNGLSEKKPADLASLLLNSAGDAQGDEAPALKLGDASC
AAPFTSKLANVSAVTNII RQGRCALVNTIQMYKILALNCLISAYSLSIIY MAGVKFGDGQ
ATVSGLLLSVCFLSISR GKPLEKLSKQRPQSGIFNVYIMGSILSQFAVHIATLVYITTEI
YKLEPREPQVDLEKEFAPSL LNTGIFI IQLVQQVSTFAVNYQGE PFRENIRSNKGMYYGL
LGV TGLALASATEFLPELNEAMKFVPMTDDFKIKLTLTLLLDFFGSWGV EHF FKF FFMDD
KPSDISVQQVKIASK

SEQ ID NO:32

YER166W

>sp|P32660|ATC5_YEAST Phospholipid-transporting ATPase DNF1
 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=DNF1
 PE=1 SV=2

MSGTFHGDGHAPMSPFEDTFQFEDNSSNEDTHIAPTHFDDGATSNKYSRPQVSFNDETPK
 NKREDAEEFTFNDDEYDNHSFQPTPKLNNGSGTFDDVELDNDSGEPHTNYDGMKRFRMG
 TKRNKKGNPIMGRSKTLKWARKNIPNPFEDFTKDDIDPGAINRAQELRTVYYNMPLPKDM
 IDEEGNPIMQYPRNKIRTTKYTPLTFLPKNILFQFHNFANVYFLVLIILGAFQIFGVNTP
 GLSAVPLVVIVIITAIKDAIEDSRRTVLDLEVNNTKTHILEGVENENVSTDNISLWRRFK
 KANSRLLFKFIQYCKEHLTEEGKKKRMQRKRHEL RVQKT VGTSGPRSSLDSIDS YRV SAD
 YGRPSLDYDNLEQGAGEANIVDRSLPPRTDCKFAKNYWKGVKVGDIVRIHNND EIPADII
 LLSTSDTDGACYVETKNLDGETNLKVRQSLKCTNTIRTSKDIARTKFWIESEGPHSNLYT
 YQGNMKWRNLADGEIRNEPITINNVLRLRGCTLRNTKWAMGVVMFTGGDTKIMLNSGITPT
 KKSRI SRELNF SVVINFVLLFILCFVSGIANGVYYDKKGRSRFSYEFGTIAGSAATNGFV
 SFWVAVILYQSLVPISLYISVEIIKTAQA AFIYGDVLLYNAKLDYPCTPKSWNISDDLQ
 VEYIFSDKTGTLTQNVMEFKKCTINGVSYGRAYTEALAGLRKRQ GIDVETEGRREKAEIA
 KDRDTMIDELRALSGNSQFYPEEVT FVSKEFVRDLKGASGEVQQRCCEHFMLALALCHSV
 LVEANPDNPKKLDLKAQSPDEAALVATARDVGFSFVGKTKKGLI IEMQGIQKEFEILNIL
 EFNSSRKRMSCIVKIPGLNPGDEPRALLICKGADSIIYSRLSRQSGSNSEAILEKTALHL
 EQYATEGLRTL CIAQRELSWSEYEK WNEKYDIAAASLANREDELEV VADSIERELILLGG
 TAIEDRLQDGV PDCIELLA EAGIKLWVLTGDKVETAINIGFSCNLLNNEMELLVIKTTGD
 DVKEFGSEPSEIVDALLSKYLKEYFNLTGSEEEIFEAKKDHEFPKGNYAIVIDGDALKLA
 LYGEDIRRKFLLLCKNCRAVLCCRVS PSQKAAVVKLVKDSLDMTLAIGDGSNDVAMIQS
 ADVGIGIAGEEGRQAVMCSDYAIGQFRYLARLVLVHGRWSYKRLAEMIPEFFYKNMIFAL
 ALFWYGIYNDFDGSYLYEYTYMMFY NLAFTSLPVI FLGILDQDVNDTISLVVPQLYRVGI
 LRKEWNQRKFLWYMLDGLYQSIICFFFPYLVYHKNMIVTSNGLGLDHR YFVG VYVTTIAV
 ISCNTYVLLHQYRWDWFSGLFIALSCLVFWA WTGIWSSAIASREFFKAAARIYGAP SFWA
 VFFVAVLFCLLPRFTYDSFQKFFYPTDVEIVRE MWQHGHFDHYPPGYDPTDPNRPKVTKA
 GQHGEKII EGIALSDNLGGSNYSRDSVVTEEIPMTFMHGEGD GSPSGYQKQETWMTSPKET
 QDLLQSPQFQQAQT FGRGPSTNVRSSLDRTREQMIATNQLDN RYSVERARTSLDLPGVTN
 AASLIGTQQNN

SEQ ID NO:33

YFL011W

>sp|P43581|HXT10_YEAST Hexose transporter HXT10 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=HXT10 PE=1 SV=1
 MVSSSVSILGTS AKASTSLSRKDEIKLTPETREASLDIPYKPIIAYWTVMGLCLMIAFGG
 FIFGWDGTISGFINQTD FKRRFGELQRDGSFQLSDVRTGLIVGIFNIGCALGGLTLGRL
 GDIYGRKIGLMCVILVYVVGIVIQIASSDKWYQYFIGRIVSGMGVGGVAVLSPTLISEIS
 PKHLRGTCVSFYQLMITLGI FLGYCTNYGTTKYSNSIQWRVPLGLCFAWAI FMVIGMVMV
 PESPRYLVEKGKYE EARRSLAKSNKVTVDPGVVFEEDTIVANMELERAVGNASWHELFS
 NKGAILPRVIMGIVIQSLQQLTGCNYFFYYGTTIFNAVGMQDSFETSIVLGAVNFASTFV
 ALYIVDKFGRRKCLLWGSASMAICFVIFATVGVTRLWPQGKDQPSSQSAGNVMIVFTCFE
 IFSFAITWAPIAYVIVAETYPLRVKNRAMAIAVGANWMWGFLIGFFTPFITRSIGFSYGY
 VFMGCLIFS YFYVFFVCETKGLTLEEVNEMYEERIKPWKSGGWI PSSR RTPQPTSSTPL
 VIVDSK

SEQ ID NO:34

YGL006W

>sp|P38929|ATC2_YEAST Calcium-transporting ATPase 2 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=PMC1 PE=1 SV=1
 MSRQDENSALLANNENNKPSYTG NENGVDNFKLSKSQLSDLHNP KSIRSFVRLFGYESN

SLFKYLKTDKNAGISLPEISNYRKTNRYKNYGDNSLPERIPKSFLQLVWAAFNDKTMQLL
 TVAAVVSFVLGLYELWMQPPQYDPEGNKIKQVDWIEGVAIMIAVFVVVLVSAANDYQKEL
 QFAKLNKKKENRKIIIVIRNDQEILISIHVVLVGDVISLQTDGVVPADCVMISSGKCEADES
 SITGESNTIQKFPVDNSLRDFKKFNSIDSHNHSKPLDIGDVNEDGNKIADCMLISGSRIL
 SGLGRGVITSVGINSVYGQTMSTLNAEPESTPLQLHLSQLADNISVYGCVSAIILFLVLF
 TRYLFYIIPEDGRFHDLDPAQKGSKF MNIFITSITVIVVAVPEGLPLAVTLALAFATTRM
 TKDGNLVRVLRSCETMGSATAVCSDKTGTLTENVMTVVRGFPGNSKFDDSKSLPVSEQRK
 LNSKKVFEENCSSSLRNDLLANIVLNSTAFENRDYKKNDKNTNGSKNMSKNLSFLDKCKS
 RLSFFKKGNREDDDEDQLFKNVNKGRQEPFIGSKTETALLSLARLSLGLQPGELQYLRDQP
 MEKFNIEKVVQTIPFESSRKWAGLVVYKKEGKNKKPFYRFFIKGAAEIVSKNCSYKRNSD
 DTLEEINEDNKKETDDEIKNLASDALRAISVAHKDFCECDSWPPEQLRDKDSPNIAALDL
 LFNSQKGLILDGLLGIQDPLRAGVRESVQQCQRAGVTVRMVTGDNILTAKAIARNCAILS
 TDISSEAYSAMEGTEFRKLTNERIRILPNLRVLARSSPEDKRLLVETLKGMDVAVTG
 DGTNDAPALKLADVGFSGMISGTEVAREASDIILMTDDFSAIVNAIKWGRCVSVSIKKFI
 QFQLIVNITAVILTFVSSVASSDETSVLTAVQLLWINLIMDTLAALALATDKPDPNIMDR
 KPRGRSTSLISVSTWKMILSQATLQLIVTFILHFYGPPELFFKKHEDEITSHQQQQNLAMT
 FNTFVWLQFFTMLVSRKLDDEGDGISNWRGRISAANLNFFQDLGRNYYFLTIMAIIGSCQV
 LIMFFGGAPFSIARQTKSMWITAVLCGMLSLIMGVLVRICPDEVAVKVFPAAFVQRFKYV
 FGLEFLRKNHTGKHDDEEALLEESDSPESTAFY

SEQ ID NO:35

YGL013C

>sp|P12383|PDR1_YEAST Transcription factor PDR1 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=PDR1 PE=1 SV=2
 MRGLTPKNGVHIETGPDTSSADSSNFSTGFSGKIRKPRSKVSKACDNCRRKIKCNGKF
 PCASCEIYSCECTFSTRQGGARIKNLHKTSLGTTVQVKEETDSSSTSFSNPQRCTDGPC
 AVEQPTKFFENFKLGGRSSGDNSGSDGKNDDDVNRNGFYEDDSESQATLTSLQTTLKNLK
 EMAHLGTHVTSIAIESIELQISDLLKRWEKPVRTKELATTKFYPNKSIETQLMKNKYCDVV
 HLTRYAAWSNNKKDQDTSSQPLIDEIFGLYSPFQFLSLQGIGKCFQNYRSKSKCEIFPRT
 AKETIYIMLRFFDVCFHINQGCVSIAENPLENYLQKMNLLPSTPSSISSAGSPNTAHTKS
 HVALVINHLPPQPFVRNITGISNSELLSEMNDISMFGILLKMLDMHKNSYQNFLMEITSN
 PSVAKNTQSIDVLQEFIHQAGEALIALCYSYNSTLYNYVDFTCDITHLEQLLYFLDL
 LFWLSEIYGFEKVLNVAVHFVSRVGLSRWEFYVGLDENFAERRRNLLWWKAFYFEKTLASK
 LGYPSNIDDSKINCLLPKNFRDVGFLDNRFIENVHLVRRSEAFDNMCISDLKYYGELAV
 LQIVSHFSSSVLFNEKFTSIRNTSKPSVVREKLLFEVLEIFNETEMKYDAIKEQTGKLFD
 IAFSKDSTELKVSREDKIMASKFVLFYEHFHRMVNESDNIVARLCVHRRPSILIENLKI
 YLHKIYKSWTDMNKILLDFDNDYSVYRSFAHYSISCIILVSQAQFSAEFIKVNDVVMIR
 VFKRFLDIKIFSENETNEHVFNSQSFKDYTRAFSFLTIVTRIMLLAYGESSSTNLDVISK
 YIDENAPDLKGIIELVLDTNSCAYRFLLEPVQKSGFHLTVSQMLKNRKFQEPLMSNEDNK
 QMKHNSGKNLNPDLPSLKTGTSCLLNGIESPQLPFNGRSAPSPVRNNSLPEFAQLPSFRS
 LSVSDMINPDYAQPTNGQNNTQVQSNKPINAQQQIPTSVQVPFMNTNEINNNNNNNNNNK
 NNINNINNNNSNNSFSATSFNLGTLDEFVNNGDLEDLYSILWSDVYPDS

SEQ ID NO:36

YGL255W

>sp|P32804|ZRT1_YEAST Zinc-regulated transporter 1 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=ZRT1 PE=1 SV=1
 MSNVTTTPWWKQWDPSEVTLADKTPDDVWKTCLVQGVYFGGNEYNGNLGARISSVFVILFV
 STFFTTFPLISTKVKRLRIPLYVYLFKAYFGSGVIVATAFIHLMDPAYGAIGGTTTCVGQT
 GNWGLYSWCPAIMLTSLTFTFLTDLFSSVWVERKYGLSHDHTHDEIKDTVVRNTAAVSSE
 NDNENGTANGSHDTKNGVEYYEDSDATSMDDVVQS FQAQFYAFLILEFGVIFHSVMIGLNL
 GSVGDEFSSLYPVLVHFHQSFEGLGIGARLSAIEFPRSKRWWPWALCVAYGLTTPICVAIG

LGVRTRYVSGSYTALVISGVLDASAGILLYTGLVELLARDFI FNPQRTKDLRELSFNVI
CTLFGAGIMALIGKWA

SEQ ID NO:37

YGR125W

>sp|P53273|YG35_YEAST Uncharacterized vacuolar membrane protein
YGR125W OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)
GN=YGR125W PE=1 SV=1

MGRTIRRRRSNSSLSEAI SVSLGINQDSSVNKMHRASVSAMSPPLCRSYMSGFFTGGNSP
MINNLSDSKLPISNKQHPKVIHGSENLHRQTAQLSNEFCSSSVEENSPTIKDYMDI IGNG
DRKDDQSMRTIEENIDEEYSDEYSRLLLSPASSNVDDDRNRGLQNSSLPELEDGYAGGYQ
SLRPSHNLRFRPRNLWHMCTSFPSKFAHYLPAAVLGLLLNILDALSYGMIIFPITEPVFS
HLGPTGISMFYISTIISQAVYSGGWSSFPSGIGSEMIEITPFYHTMALAIKEALAGNDDE
IITTTIFCYVISSMLTG VVFYALGKLR LGKIVGFFPRHILIGCIGGVGYFLIITGIEVTT
RVAKFEYSWPFFSGLFTDYDTLAKWLLPVLLTVVLIGTQRYFKNSLVLP SFYILT LVLFH
FIVAIIPTLSLDALRQAGWIFPIANSDSKWYDHYRLFNVHKVHWSLV LQQIPTMMALTFF
GILHVPINVPALAMSLQMDKYDVDRELIAHGYSNFFSGLLGSVQNYLVYTNSVLFIRAGA
DSPFAGFLLIALTICIMIIGPVIISFIPICIVGSLIFLLGYELLVEALVDTWNKLNRF EY
LTVVIIIVFTMGIFDFVLGIIVGILIA CFSFLVDSTKLQTINGEYNGNVARSTVYRDYVQT
KFLDGIGE QIYVLKLQNL LFFGTIISIEEKIERLLQISNKDATKRRIKYLILDFKNINAD
NIDYSAAEGFNRIKRFTETKRIKLI ISSIKERDRIYNAFN NVGLLNDVELFADLNSALEW
CENEFLFQYKQLRKKAKERLEEGKQNNVVS AVIAATKNKKIDTIGNGLNRGSNGDTARNL
MSLPTNTPRNYQILSVAQNVFVNDEQAVKNFKKEYKDDEPVLPI LLFALKQYRPDI ISEV
QKVREKEIKFWAQLCPYFTRRRLASQSHLLHADNIFFLVETGMLKATYELPQGTLYEIFS
NGTCFGKIIAPGNAMPREQKLT IETETDSVLWVIDSSSLNKLKEDNLALYVEVALMVMCI
KDTRFKELLGYTLVSA

SEQ ID NO:38

YGR181W

>sp|P53299|TIM13_YEAST Mitochondrial import inner membrane
translocase subunit TIM13 OS=Saccharomyces cerevisiae (strain ATCC
204508 / S288c) GN=TIM13 PE=1 SV=1
MGLSSIFGGGAPSQQKEAATTAKTTPNPIAKELKNQIAQELAVANATELVN KISENCFEK
CLTSPYATRNDACIDQCLAKYMRSWNVISKAYISRIQNASASGEI

SEQ ID NO:39

YGR217W

>sp|P50077|CCH1_YEAST Calcium-channel protein CCH1 OS=Saccharomyces
cerevisiae (strain ATCC 204508 / S288c) GN=CCH1 PE=1 SV=1
MQGRKRTLTEPFEPNTNPFGDNAAVMTENVEDNSETDGNRLESKPQALVPPALNIVPPES
SIHSTEEKKGDEYNGNDKDSSLISNIFRTRVGRSSHENLSRPKLSLKTASFGAAESSRRN
VSPSTKSAKSSSQYIDLNDERLRRRSFSSYSRSSSRVSNSPSSTDRPPRS AKVLSLIAA
DDMDDFEDLQKGFKSAIDEEGLTWLPQLKSEKSRPVSDVGEDRGEGEQESI PDVHTPNVG
ASATPGSIHLTPEPAQNGSVSEGLEGSINNSRKKPSPKFFHHLSPQKEDKDQTEVIEYAE
DILDFETLQRKLESRPFVLYGHSLGVFSPTNPLRIKIARFLLHRRYSLLYNTLLTFYAIL
LAIRTYNPHNVVFLYRFSNWTDYFIFILSACFTGNDIAKIIAFGFWD DSEMFKAYGREYK
SILQRSGIMKLYIYLREKYGRKLIDFII PFRIISPGEETKYQRSSLSTSLTKPYGAKENQ
RPFGT PRA FARSSWNRIDLVSSVSFWLGMFLSIKSYDTKTGIRIFKPLAILRILRLVNVD
TGMPSILRGLKYGIPQLVNVSSMLVYFWIFFGILGVQIFQGSFRRQCVWFNPEDPTDTYQ

YDMQFCGGYLDPVTKRKQNYIYEDGSEGSVSKGFLCPQYSKCVSNANPYNGRISFDNIVN
 SMELVFVIMSANTFTDLMYYTMDSDEMAACLFIVCIVLTIWLLNLLIAVLVSSFEIAN
 EEEYKKKKFIYGSRKTYGYVARIVTGYWKYFKLKANQTKFPNWSQKGLAIYSHVEFIFVILI
 ICDIGMRASVKVSTSANCNILLKTDRGISIVLFIESLARLVLYLPNMWKFLTTPSYVYD
 FIISIITLVISCLAVEGVLGHMYAWLSIFHISRFRYRVIIISFNLTKKLWKQILSNGVMIWN
 LSSFYFFFTFLVAIIMAVYFEGVIPPEEMADQPFMYSLPNSFLSLFIIIGSTENWTDILY
 ALQKHSPNISSTFFCSVFFIIWFLLSNSVILNIFIALISESMEVKEEEKRPQQIKHYLKF
 VYPQKIQEYTHASLVARIRKKFFGGHRNEDTRDFKQFLMRGTAIMNIAQNMGELADEFKE
 PPSENLFKKGLSKLTIGVPSLKRRLRMFANNPFYKNSDVVFTETNDINGRTYILELNEYED
 EKLDYLKKYPLFNYSYYFFSPQHRFRRFCQRLVPPSTGKRTDGSRRFFEDSTDLYNKRSYF
 HHIERDVVFVIFALATILLIVCSCYVTPLYRMHHKMGWTWNWSSALDCAFIGAFSIEFIVK
 TVADGFIYSPNAYLRNPWNFIDFCVLISMWINLIAYLKNNGNLSRIFKGLTALRALRCLT
 ISNTARQTFNLVMFDGLNKIFEAGLISLSLLFPFTVWGLSIFKGRLGTCNDGSLGRADCY
 NEYSNSVQWDIMSPRVYQQPYLHLD SFASAFSSLYQIISLEGWVDLLENMMNSSGIGTP
 ATVMGSAGNALFLVLNLFSLMVFILNLFVSFIVNNQARTTGSAYFTIEEKAWLESQKLLS
 QAKPKAIPNLIELSRVRQFFYQLAVEKKNFYASFLQVVLYLHIIMLLSRSYNPGNLIGY
 QGVYFMFSTSVFLIQEALHMCGEPRLYFRQKWN SIRLSIIIIAFIMNAVAFHVPASHYW
 FHNIKGFFLLVIFLFIIPQNDTLTELLETAMASLPPILSLTYTWGVFLVYAIALNQIFG
 LTRLGSNTTDNINFRTVIKSMIVLFRCSFGEGWNYIMADLTVSEPYCSSDDNSTYTDCGS
 ETYAYLLLSWNIISMYIFVNMVSLIIGNFSYVYRSGGSRSGINRSEIKKYIEAWSKFD
 TDGTGELELSYLPRIMHSFDGPLSFKIWEGRLTIKSLVENYMEVNPDDPYDVKIDLIGLN
 KELNTIDKAKIIQRKLQYRRFVQSIHYTNAYNGCIRFSDLLLQIPLYTAYSARECLGIDQ
 YVHHLYILGKVDKYLENQRNFDVLEMVTRWKFHCRMKRTIEPEWDVKDPTVSSHISNIN
 VNLEPAPGILEREPIATPRMDYGVNNFMWSPRMNQDSTMEPPEEPIDNNDDSANDLIDR

SEQ ID NO:40

YGR224W

>sp|P50080|AZR1_YEAST Azole resistance protein 1 OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=AZR1 PE=1 SV=1
 MKGEPKTYSMSDLSYYGEKAQQQNEKQQKQYVVRNSTQSTSKQNVSVVLEDNASESNEL
 PKGFILYASLIALALSFLAALDIMIVSTIIIEEVAKQFGSYSEIGWLFTGYSLPNALLAL
 IWGRIATPIGFKETMLFAIVIFEIGSLISALANSMSMLIGGRVIAGVGGCGIQSLSFVIG
 STLVEESQRGILIAVLSCSFAIASVVGPFLLGGVFTSSVTWRWCFYVNLPIGGLAFFLFLF
 FYNPGLSTFQETMDNIRKFPSQFIEIVRNVAYHLLKIKGFSKLNWGRKPFMELIFMYDII
 EFVFCASAGFTCILLAFTFGGNRYAWNSASIIILFIIGIVLVVLAGIYDFLVFPKFNIVKA
 TPHYQPLMSWTNIKKPGIFTVNIALFLTCAGYISQFTYIVQYFQLIYNDSAWRAAVHLVA
 CIISTVVTAILCGAITDKTRQIKPIIIVISSIFGVVGAGILTLLNNNANNSAHIGLLILPG
 VAFGGLAQSSMLASQIQLDKKSPTFRSDFVSITTFNTFCKNLGQALGGVISNTVFSAAAI
 KKLTKANIQLPDGTTVDNLVIYRQTNFDGSHSKLGNIISESLTDVFYMALGFYALSLIFA
 VFASNKKVTASLR

SEQ ID NO:41

YGR281W

>sp|P53049|YOR1_YEAST Oligomycin resistance ATP-dependent permease
 YOR1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)
 GN=YOR1 PE=1 SV=1
 MTITVGDAVSETELENKSQNVVLSPKASASSDISTDVKDTSSSWDDKSLLPTGEYIVDR
 NKPQTYLNSDDIEKVTESDIFPQKRLFSFLHSKKIPEVPQTDDERKIYPLFHTNIISNMF
 FWWVLPILRVGYKRTIQPNDLFKMDPRMSIETLYDDFEKNMIYYFEKTRKKYRKRHPEAT
 EEEVMENAKLPKHTVLRALLFTFKKQYFMSIVFAILANCTSGFNPMITKRLIEFVEEKAI
 FHSMHVNGGIGYAIGACLMMFVNGLTFNHFFHTSQLTGVOAKSILTKAAMKKMFNASNYA

RHCFPNGKVTSEFVTTDLARIEFALSFQPFFLAGFPAILAICIVLLIVNLGPIALVIGIGIFF
 GGFFISLFAFKLILGFRIAANIFTDARVTMMREVLNNIKMIKYYTWEDAYEKNIQDIRTK
 EISKVRKMQLSRNFLIAMAMSLPSIASLVTF LAMYKVNKGGRQPGNIFASLSLFQVLSLQ
 MFFLPPIAIGTGIDMIIGLGRLQSLLEAPEDDPNQMIEMKPSPGFDPKLALKMTHCSFEWE
 DYELNDAIEEAKGEAKDEGKKNKKRKRDTWGKPSASTNKA KRLDNMLKDRDGPEDLEKTS
 FRGFKDLNFDIKKGEFIMITGPIGTGKSSLLNAMAGSMRKTGDKVEVNGDLLMCGYPWIQ
 NASVRDNIIFGSPFNKEYDEVVRVCSLKADLDILPAGDMTEIGERGITLSGGQKARINL
 ARSVYKKKDIYLFDDVLSAVDSRVGKHIMDECLTGMLANKTRILATHQLSLIERASRVIV
 LGTDGQVDIGTVDELKARNQTLINLLQFSSQNSEKEDEEQEAVVAGELGQLKYESEVKEL
 TELKKKATEMSQTANSGKIVADGHTSSKEERAVNSISLKIYREYIKA AVGKWGFIALPLY
 AILVVGTTFCSLFSSVWLSYWTENKFKNRPPSFYMGLYSFFVFAAFIFMNGQFTILCAMG
 IMASKWLNLRVAVKRILHTPMSYIDTTPLGRILNRFTKDTDSLDELTESLRLMTSQFANI
 VGVCVMCIVYLPWFAIAIPFLLVIFVLIADHYQSSGREIKRLEAVQRSFVYNNLNEVLGG
 MDTIKAYRSQERFLAKSDFLINKMNEAGYLVVVLQRWVGIFLDMVAIAFALIITLLCVTR
 AFPISAASVGVLLTYVLQLPGLLNTILRAMTQTENDMNSAERLVTYATELPLEASYRKPE
 MTPPESWPSMGEIIFENVDFAYRPGLPIVLKNLNLNLIKSGEKIGICGRTGAGKSTIMSAL
 YRLNELTAGKILIDNVDISQLGLFDLRRKLAIIPQDPVLFRGTIRKNLDPFNERTDDELW
 DALVRGGAIKDDLPEVKLQKPDENGTHGKMHKFHLDQAVEEEGSGNFSLGERQLLALTRA
 LVRQSKILILDEATSSVDYETDGKIQTRIVEEFGDCTILCIAHRLKTIVNYDRILVLEKG
 EVAEFDTPWTLFSQEDSIFRSMCSRSGIVENDFENRS

SEQ ID NO:42

YHL016C

>sp|P33413|DUR3_YEAST Urea active transporter OS=Saccharomyces
 cerevisiae (strain ATCC 204508 / S288c) GN=DUR3 PE=1 SV=2
 MGEFKPPLPQGAGYAIVLGLGAVFAGMMVLTTYLLKRYQKEIITAEFFTAGR SVKTGLV
 AA AVVSSWIWCSTLLTSSTKEYADGIFGGYAYAAGACFQIIAFAILAIKTKQMAPNAHTY
 LELVRTRYGKIGHGCVLFYAIATNILVTSMLLTSGSAVFSDLTGMENTIASCFLLPVGVVV
 YTLFGGIKATFLTDYMHTCVIIIIIVLVFAFKVYATSDVLGSPGKVYDLVREAAKRHPVDG
 NYQGEYMTMTSKSAGILLIINLIGNFGTVFLDNGYWNKAISASPAASLKAYAIGGLAWFA
 VPSLISLTMGLACLAVETSPNFPTYPDPLTSFQANSGLVLPAAAI AIMGKGGAVASLLMI
 FMAVTSAMSAELIAVSSVFTYDIYREYIDPRASGKKLIYTSHVACIFFGLAMSGFSVGLY
 YGGISMGYIYEMMGIIISSAVLPVVLTLCSKDMNLVAAVVSPI LGTGLAIMSWLVCTKSL
 YKELTVDTTFMDYPMLTGNLVALLSPAIFIPILTYVFKPQNF DWEKMKDITRVDETAELV
 QADPDIQLYDAEANDKEQEEETNSLVSDSEKNDVRVNNEK LIEPNLGVVISNAIFQEDDT
 QLQNELDEEQRELARGLKIA YFLCVFFALAFLLVWVWPMMPMYGSKYIFSKKFFTGWVVVMII
 WLFSAFAVCIYPLWEGRHGIYTTLRGLYWDL SGQTYKLREWQNSNPQDLHVVT SQISAR
 AHRQSSHFGQVDEII

SEQ ID NO:43

YIL088C

>sp|P40501|AVT7_YEAST Vacuolar amino acid transporter 7
 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=AVT7
 PE=1 SV=1
 MEATSSALSSTANLVKTIVGAGTLAIPYSFKSDGVLVGVILTLLAAVTSGLG LFLVLSKCS
 KTLINPRNSSFFTL CMLTYPTLAPIFDLAMIVQC FGVGLSYLVLIGDLFPGLFGGER NYW
 IIASAVIIIP LCLVKKLDQLKYSSILGLFALAYISILVFSHFV FELGKGELTNILRNDIC
 WWKIHFDFKGLLSTFSIIIF AFTGSMNLFPMINELKDNSMENITFVINNSISLSTALFLIV
 GLSGYLTFGNETLGNLMLNYDPNSIWIVIGKFCLG SMLILSFPLL FHLPLRIAVNNV IWI
 EITYGGANPEEDPQVSEYTRASNL RPISMTVEDPAQPSDALDATSYNEQECLLPNGNFDN
 GSIESQENNNDERGTMAVAGDNEHHAPFVKSRFYWITALLLISMYTLALSVQS FALVLSF

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 78

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 78

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A recombinant host capable of synthesizing a steviol glycoside, comprising a gene encoding a transporter polypeptide and/or a gene encoding a transcription factor polypeptide that regulates expression of at least one transporter gene;

wherein expression of the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide that regulates expression of at least one transporter gene is modified and the recombinant host transports at least a portion of the synthesized steviol glycoside from the host into a culture medium.
2. The recombinant host of claim 1, wherein the gene encoding the transporter polypeptide is an endogenous gene.
3. The recombinant host of any one of claims 1 or 2, wherein the transporter polypeptide comprises an ATP-binding cassette (ABC) transporter, a major facilitator superfamily (MFS) transporter, an amino acid/auxin permease (AAP) family transporter, an ATPase transporter, a sulfate permease (SulP) family transporter, a lysosomal cystine transporter (LCT) family transporter, a Ca^{2+} :cation antiporter (CaCA) family transporter, an amino acid-polyamine-organocation (APC) superfamily transporter, a multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) transporter, a ZRT/IRT-like protein (ZIP) metal transporter family transporter, a mitochondrial protein translocase (MPT) family transporter, a voltage-gated ion channel (VIC) family transporter, a monovalent cation:proton antiporter-2 (CPA2) family transporter, a ThrE family of putative transmembrane amino acid efflux transporter, an oligopeptide transporter (OPT) family transporter, a K^{+} transporter (Trk) family transporter, a bile acid:Na symporter (BASS) family transporter, a drug/metabolite transporter (DMT) superfamily transporter, a mitochondrial carrier (MC) family transporter, an auxin efflux carrier (AEC) family transporter, an ammonia channel transporter (Amt) family transporter, a metal ion (Mn^{2+} -iron) transporter (Nramp) family transporter, a transient receptor potential Ca^{2+} channel (TRP-CC) family transporter, an arsenical resistance-3 (ACR3) family transporter, a nucleobase:cation symporter-1 (NCS1) family transporter, an inorganic phosphate transporter (PiT) family transporter, an arsenite-antimonite (ArsAB) efflux family transporter, an IISP family of transporter, a glycerol uptake (GUP) family transporter, a metal ion transport

(MIT) family transporter, a copper transport (Ctr) family transporter, or a cation diffusion facilitator (CDF) family transporter.

4. The recombinant host of any one of claims 1-3, wherein modified expression comprises:
 - (a) overexpressing the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide; or
 - (b) deleting the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide.
5. The recombinant host of any one of claims 1-4, wherein the gene encoding the transporter polypeptide and/or the gene encoding the transcription factor polypeptide has an activity that is increased.
6. The recombinant host of any one of claims 1-5, wherein one or more of the genes encoding the transporter polypeptide and/or one or more of the gene encoding the transcription factor polypeptide is overexpressed.
7. The recombinant host of claim 6, wherein the transporter polypeptide and/or transcription polypeptide comprise YAL067C set forth in SEQ ID NO:14, YBL089W set forth in SEQ ID NO:15, YBL099W set forth in SEQ ID NO:16, YBR008C set forth in SEQ ID NO:86, YBR021W set forth in SEQ ID NO:87, YBR043C set forth in SEQ ID NO:88, YBR180W set forth in SEQ ID NO:13, YBR241C set forth in SEQ ID NO:17, YBR287W set forth in SEQ ID NO:89, YBR294W set forth in SEQ ID NO:18, YBR295W set forth in SEQ ID NO:90, YBR296C set forth in SEQ ID NO:91, YCL038C set forth in SEQ ID NO:92, YCL069W set forth in SEQ ID NO:19, YCR011C set forth in SEQ ID NO:93, YCR028C set forth in SEQ ID NO:20, YCR075C set forth in SEQ ID NO:21, YDL054C set forth in SEQ ID NO:94, YDL100C set forth in SEQ ID NO:95, YDL128W set forth in SEQ ID NO:22, YDL185W set forth in SEQ ID NO:23, YDL194W set forth in SEQ ID NO:24, YDL210W set forth in SEQ ID NO:25, YDL245C set forth in SEQ ID NO:96, YDL247W set forth in SEQ ID NO:97, YDR011W set forth in SEQ ID NO:98,

YDR061W set forth in SEQ ID NO:26, YDR093W set forth in SEQ ID NO:27, YDR292C set forth in SEQ ID NO:99, YDR338C set forth in SEQ ID NO:28, YDR406W set forth in SEQ ID NO:29, YDR497C set forth in SEQ ID NO:100, YDR536W set forth in SEQ ID NO:30, YEL006W set forth in SEQ ID NO:101, YEL027W set forth in SEQ ID NO:102, YEL031W set forth in SEQ ID NO:31, YEL065W set forth in SEQ ID NO:103, YER019C-A set forth in SEQ ID NO:104, YER053C set forth in SEQ ID NO:105, YER119C set forth in SEQ ID NO:106, YER166W set forth in SEQ ID NO:32, YFL011W set forth in SEQ ID NO:33, YFL028C set forth in SEQ ID NO:107, YFR045W set forth in SEQ ID NO:108, YGL006W set forth in SEQ ID NO:34, YGL013C set forth in SEQ ID NO:35, YGL084C set forth in SEQ ID NO:109, YGL104C set forth in SEQ ID NO:110, YGL114W set forth in SEQ ID NO:111, YGL167C set forth in SEQ ID NO:112, YGL255W set forth in SEQ ID NO:36, YGR125W set forth in SEQ ID NO:37, YGR181W set forth in SEQ ID NO:38, YGR217W set forth in SEQ ID NO:39, YGR224W set forth in SEQ ID NO:40, YGR257C set forth in SEQ ID NO:113, YGR281W set forth in SEQ ID NO:41, YHL016C set forth in SEQ ID NO:42, YHL035C set forth in SEQ ID NO:114, YHL036W set forth in SEQ ID NO:115, YHR002W set forth in SEQ ID NO:116, YHR096C set forth in SEQ ID NO:117, YIL006W set forth in SEQ ID NO:118, YIL088C set forth in SEQ ID NO:43, YIL120W set forth in SEQ ID NO:119, YIL121W set forth in SEQ ID NO:120, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJL094C set forth in SEQ ID NO:45, YJL108C set forth in SEQ ID NO:46, YJL133W set forth in SEQ ID NO:122, YJL212C set forth in SEQ ID NO:47, YJL219W set forth in SEQ ID NO:123, YJR106W set forth in SEQ ID NO:48, YJR160C set forth in SEQ ID NO:49, YKL016C set forth in SEQ ID NO:124, YKL050C set forth in SEQ ID NO:125, YKL064W set forth in SEQ ID NO:50, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKL209C set forth in SEQ ID NO:128, YKR039W set forth in SEQ ID NO:129, YKR050W set forth in SEQ ID NO:51, YKR105C set forth in SEQ ID NO:52, YKR106W set forth in SEQ ID NO:53, YLR411W set forth in SEQ ID NO:130, YLR447C set forth in SEQ ID NO:54, YML038C set forth in SEQ ID NO:131, YML116W set forth in SEQ ID NO:55, YMR034C set forth in SEQ ID NO:56, YMR056C set forth in SEQ ID NO:57, YMR166C set forth in SEQ ID NO:132, YMR253C set forth in SEQ ID NO:58, YMR279C set forth in SEQ ID NO:133, YNL003C set forth in SEQ ID NO:134, YNL065W set forth in SEQ ID NO:59, YNL070W set forth in SEQ ID NO:60, YNL083W set forth in SEQ ID NO:61, YNL095C set forth in SEQ ID NO:62, YNL121C set forth in SEQ ID NO:63, YNL142W set forth in SEQ ID NO:64, YNL268W set forth in SEQ ID NO:135,

YNR055C set forth in SEQ ID NO:136, YOL020W set forth in SEQ ID NO:65, YOL075C set forth in SEQ ID NO:66, YOL077W-A set forth in SEQ ID NO:67, YOL122C set forth in SEQ ID NO:68, YOL158C set forth in SEQ ID NO:137, YOR079C set forth in SEQ ID NO:69, YOR087W set forth in SEQ ID NO:70, YOR092W set forth in SEQ ID NO:71, YOR100C set forth in SEQ ID NO:138, YOR130C set forth in SEQ ID NO:72, YOR153W set forth in SEQ ID NO:139, YOR222W set forth in SEQ ID NO:73, YOR271C set forth in SEQ ID NO:140, YOR273C set forth in SEQ ID NO:141, YOR291W set forth in SEQ ID NO:74, YOR306C set forth in SEQ ID NO:75, YOR307C set forth in SEQ ID NO:142, YOR316C set forth in SEQ ID NO:76, YOR332W set forth in SEQ ID NO:143, YOR334W set forth in SEQ ID NO:77, YOR348C set forth in SEQ ID NO:144, YPL036W set forth in SEQ ID NO:145, YPL078C set forth in SEQ ID NO:78, YPL270W set forth in SEQ ID NO:79, YPL274W set forth in SEQ ID NO:80, YPR003C set forth in SEQ ID NO:81, YPR011C set forth in SEQ ID NO:82, YPR058W set forth in SEQ ID NO:83, YPR128C set forth in SEQ ID NO:84, or YPR201W set forth in SEQ ID NO:85.

8. The recombinant host of any one of claims 1-7, wherein YBR043C set forth in SEQ ID NO:88, YDL100C set forth in SEQ ID NO:95, YDL054C set forth in SEQ ID NO:94, YDL128W set forth in SEQ ID NO:22, YDL198C set forth in SEQ ID NO:146, YDR061W set forth in SEQ ID NO:26, YDR536W set forth in SEQ ID NO:30, YEL027W set forth in SEQ ID NO:102, YFL054C set forth in SEQ ID NO:147, YGL167C set forth in SEQ ID NO:112, YGR181W set forth in SEQ ID NO:38, YHL016C set forth in SEQ ID NO:42, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJR106W set forth in SEQ ID NO:48, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKR039W set forth in SEQ ID NO:129, YMR034C set forth in SEQ ID NO:56, YMR166C set forth in SEQ ID NO:132, YOL122C set forth in SEQ ID NO:68, YOR079C set forth in SEQ ID NO:69, YPL270W set forth in SEQ ID NO:79, and/or YPR011C set forth in SEQ ID NO:82 are overexpressed.
9. The recombinant host of any one of claims 1-8, further comprising:
 - (a) one or more genes encoding a sucrose transporter and a sucrose synthase;

- (b) a gene encoding a geranylgeranyl diphosphate synthase (GGPPS) polypeptide;
- (c) a gene encoding an ent-copalyl diphosphate synthase (CDPS) polypeptide;
- (d) a gene encoding a kaurene synthase (KS) polypeptide;
- (e) a gene encoding a kaurene oxidase (KO) polypeptide;
- (f) a gene encoding a steviol synthase (KAH) polypeptide;
- (g) a gene encoding a cytochrome P450 reductase (CPR) polypeptide;
- (h) a gene encoding a UGT85C2 polypeptide;
- (i) a gene encoding a UGT76G1 polypeptide;
- (j) a gene encoding a UGT74G1 polypeptide;
- (k) a gene encoding a UGT91D2 functional homolog; and/or
- (l) a gene encoding a EUGT11 polypeptide;

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

wherein the host is capable of producing one or more of RebA, RebB, RebD and/or RebM.

10. The recombinant host of claim 9, wherein at least one of the genes is codon optimized for expression in the host.
11. The recombinant host of claim 10, wherein at least one of the genes is codon optimized for expression in *Saccharomyces cerevisiae*.
12. The recombinant host of claim 9, wherein:
 - (a) the GGPPS polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:149;
 - (b) the CDPS polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:150;

- (c) the KO polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:152;
 - (d) the KS polypeptide comprises a polypeptide having at least 40% identity to an amino acid sequence set forth in SEQ ID NO:151;
 - (e) the KAH polypeptide comprises a polypeptide having at least 60% identity to an amino acid sequence set forth in SEQ ID NO:154;
 - (f) the CPR polypeptide comprises a polypeptide having at least 70% identity to an amino acid sequence set forth in SEQ ID NO:153 and/or a polypeptide having at least 65% identity to an amino acid sequence set forth in SEQ ID NO:155;
 - (g) the UGT85C2 polypeptide comprises a polypeptide having at least 55% identity to an amino acid sequence set forth in SEQ ID NO:156;
 - (h) the UGT76G1 polypeptide comprises a polypeptide having at least 50% identity to an amino acid sequence set forth in SEQ ID NO:158;
 - (i) the UGT74G1 polypeptide comprises a polypeptide having at least 55% identity to an amino acid sequence set forth in SEQ ID NO:157;
 - (j) the a UGT91D2 functional homolog comprises a UGT91D2e-b polypeptide having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:159; and
 - (k) the EUGT11 polypeptide comprises a polypeptide having at least 65% identity to an amino acid sequence set forth in SEQ ID NO:148.
13. The recombinant host of any one of claims 1-12, wherein the recombinant host comprises a microorganism that is a plant cell, a mammalian cell, an insect cell, a fungal cell, or a bacterial cell.
14. The recombinant host of claim 13, wherein the bacterial cell comprises *Escherichia* bacteria cells, *Lactobacillus* bacteria cells, *Lactococcus* bacteria cells, *Cornebacterium* bacteria cells, *Acetobacter* bacteria cells, *Acinetobacter* bacteria cells, or *Pseudomonas* bacterial cells.
15. The recombinant host of claim 13, wherein the fungal cell is a yeast cell.

16. The recombinant host of claim 15, wherein the yeast cell is a cell from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Candida glabrata*, *Ashbya gossypii*, *Cyberlindnera jadinii*, *Pichia pastoris*, *Kluyveromyces lactis*, *Hansenula polymorpha*, *Candida boidinii*, *Arxula adeninivorans*, *Xanthophyllomyces dendrorhous*, or *Candida albicans* species.
17. The recombinant host of claim 16, wherein the yeast cell is a *Saccharomycete*.
18. The recombinant host of claim 17, wherein the yeast cell is a cell from the *Saccharomyces cerevisiae* species.
19. A method of producing a steviol glycoside, comprising:
 - (a) growing the recombinant host of any one of claims 1-18 in a culture medium, under conditions in which the genes discussed in any one of claims 1 to 18 are expressed,

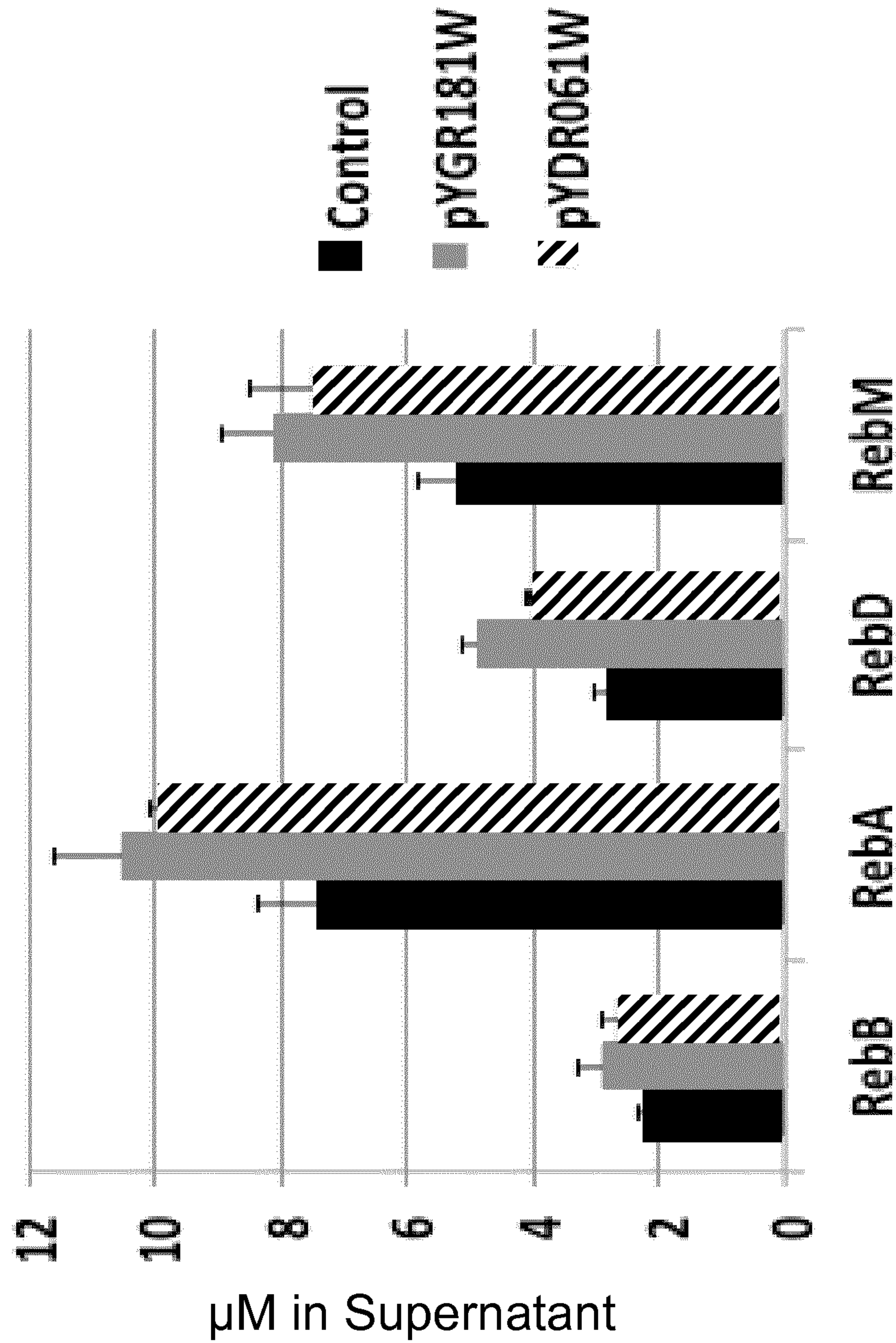
wherein the steviol glycoside is synthesized by the host; and
 - (b) optionally isolating the steviol glycoside.
20. The method of claim 19, wherein the steviol glycoside is RebA, RebB, RebD, and/or RebM, and wherein:
 - (a) RebA is capable of being synthesized in the recombinant host of any one of claims 1-18 expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2;
 - (b) RebB is capable of being synthesized in the recombinant host of any one of claims 1-18 expressing UGT85C2, UGT76G1, and UGT91D2;
 - (c) RebD is capable of being synthesized in the recombinant host of any one of claims 1-18 expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11; and
 - (d) RebM is capable of being synthesized in the recombinant host of any one of claims 1-18 expressing UGT85C2, UGT76G1, UGT74G1, and UGT91D2 and/or EUGT11.

21. The method of claims 19 or 20, wherein a gene encoding YBR043C set forth in SEQ ID NO:88, YDL100C set forth in SEQ ID NO:95, YDL054C set forth in SEQ ID NO:94, YDL128W set forth in SEQ ID NO:22, YDL198C set forth in SEQ ID NO:146, YDR061W set forth in SEQ ID NO:26, YDR536W set forth in SEQ ID NO:30, YEL027W set forth in SEQ ID NO:102, YFL054C set forth in SEQ ID NO:147, YGL167C set forth in SEQ ID NO:112, YGR181W set forth in SEQ ID NO:38, YHL016C set forth in SEQ ID NO:42, YIL166C set forth in SEQ ID NO:121, YJL093C set forth in SEQ ID NO:44, YJR106W set forth in SEQ ID NO:48, YKL120W set forth in SEQ ID NO:126, YKL146W set forth in SEQ ID NO:127, YKR039W set forth in SEQ ID NO:129, YMR034C set forth in SEQ ID NO:56, YMR166C set forth in SEQ ID NO:132, YOL122C set forth in SEQ ID NO:68, YOR079C set forth in SEQ ID NO:69, YPL270W set forth in SEQ ID NO:79, and/or YPR011C set forth in SEQ ID NO:82 is overexpressed.
22. The method of any one of claims 19-21, wherein the steviol glycoside is produced at a concentration of between about 500 mg/L to about 10,000 mg/L.
23. A method of increasing production or transport of a steviol glycoside into a culture medium, comprising:
- (a) growing the recombinant host of any one of claims 1-18 in a culture medium, under conditions in which the genes discussed in any one of claims 1 to 18 are expressed,
wherein the steviol glycoside is synthesized by the host; and
 - (b) optionally isolating the steviol glycoside.
24. The method of claim 23, wherein the steviol glycoside is RebA, RebB, RebD, and/or RebM.

1/11



Figure 2



3/11

Figure 3A

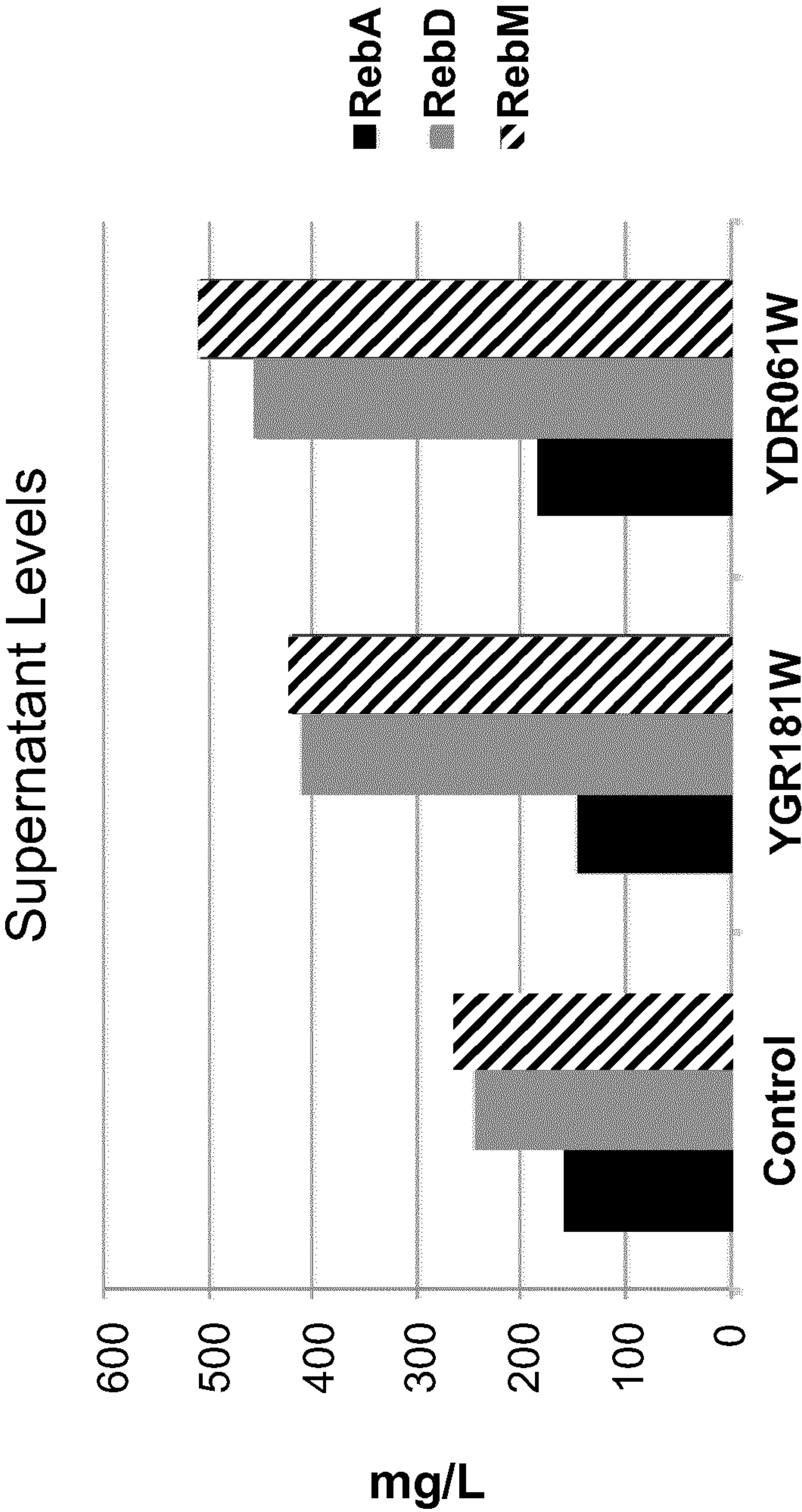


Figure 3B

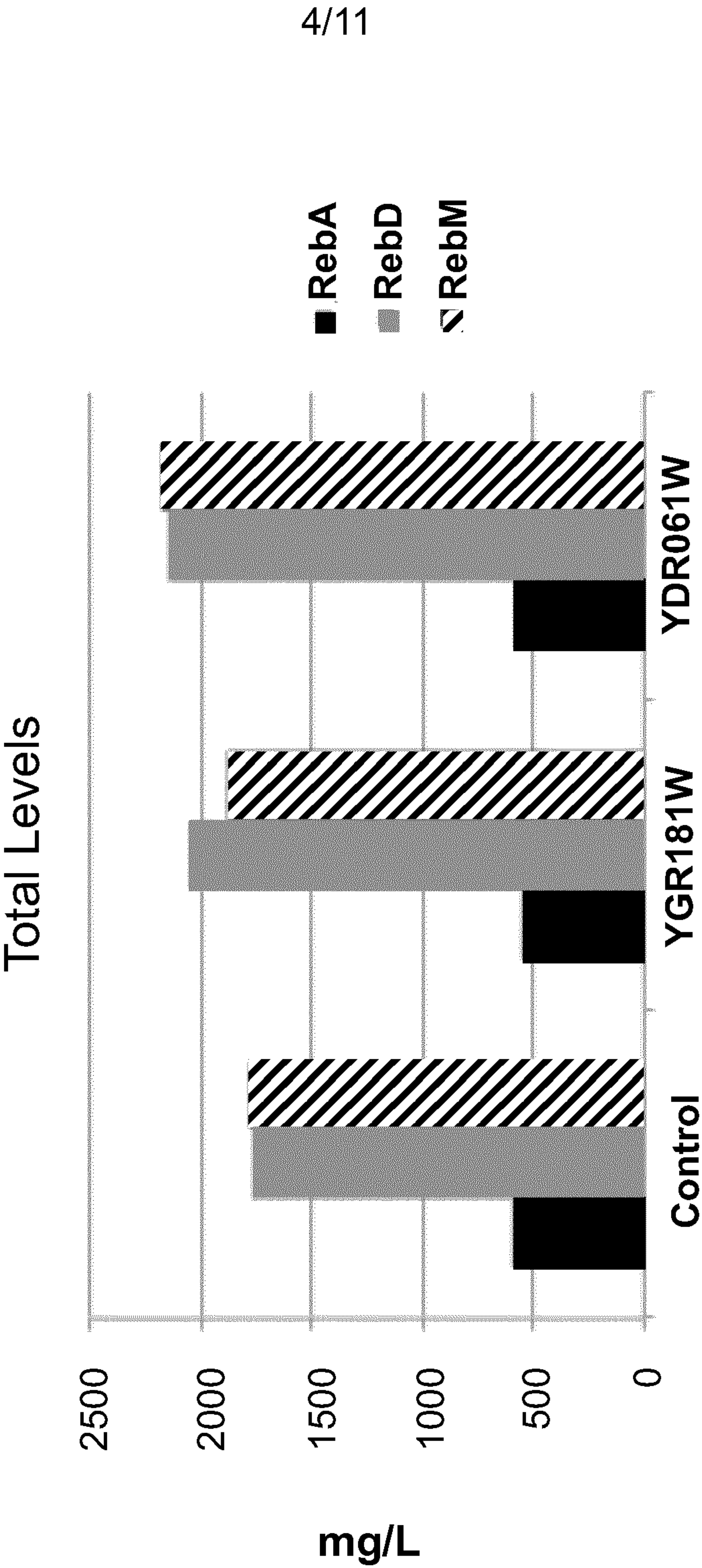


Figure 4A
13-SMG

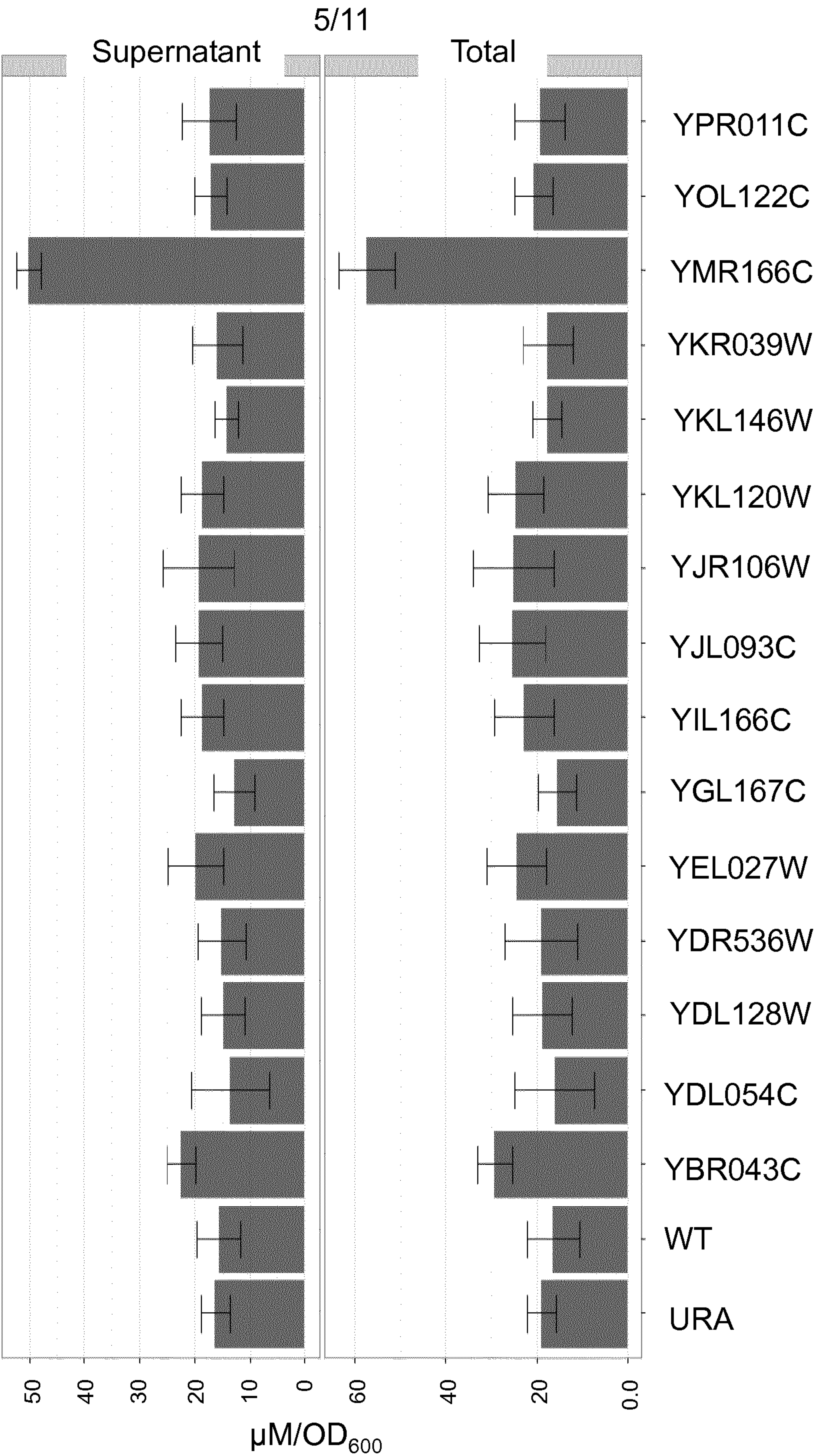


Figure 4B
RebA

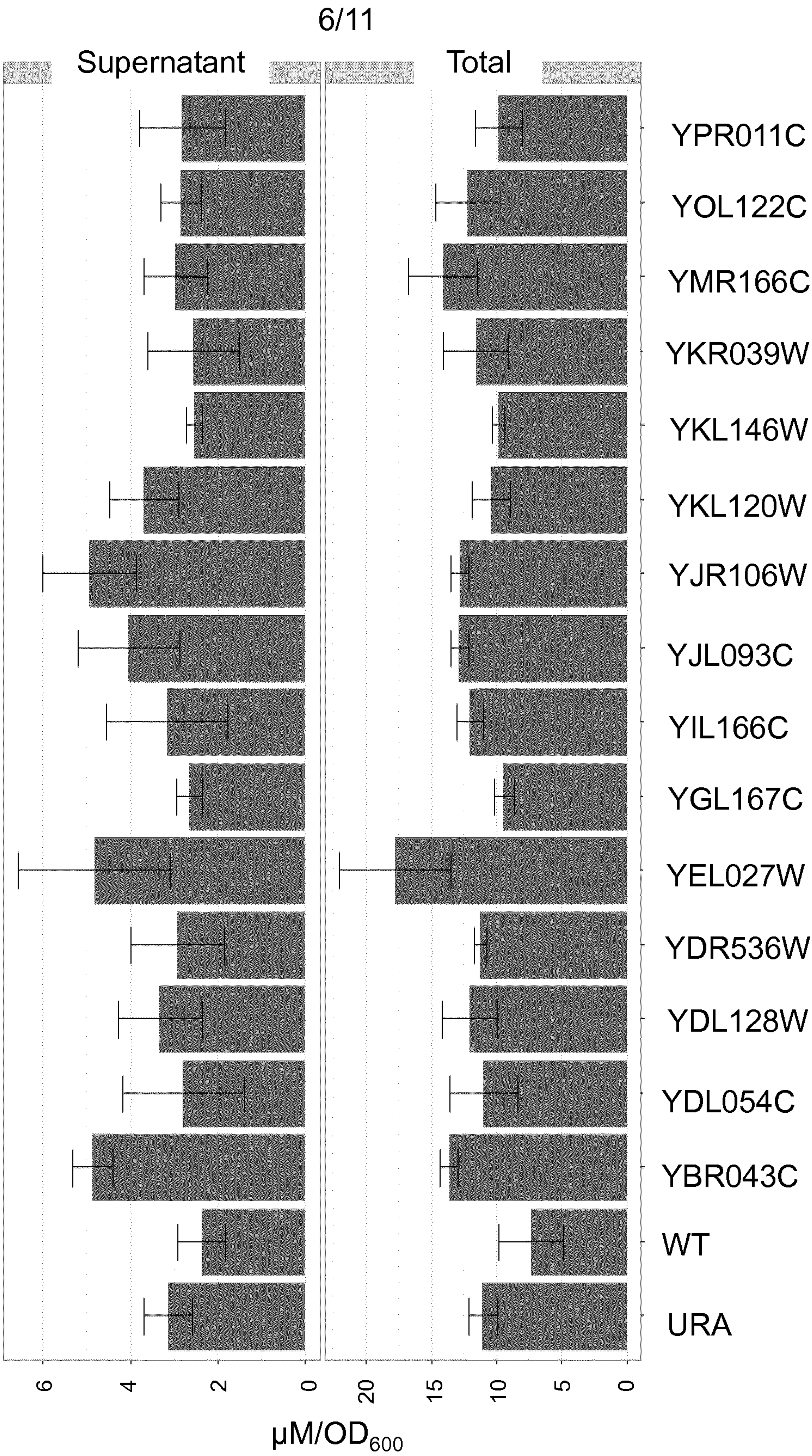
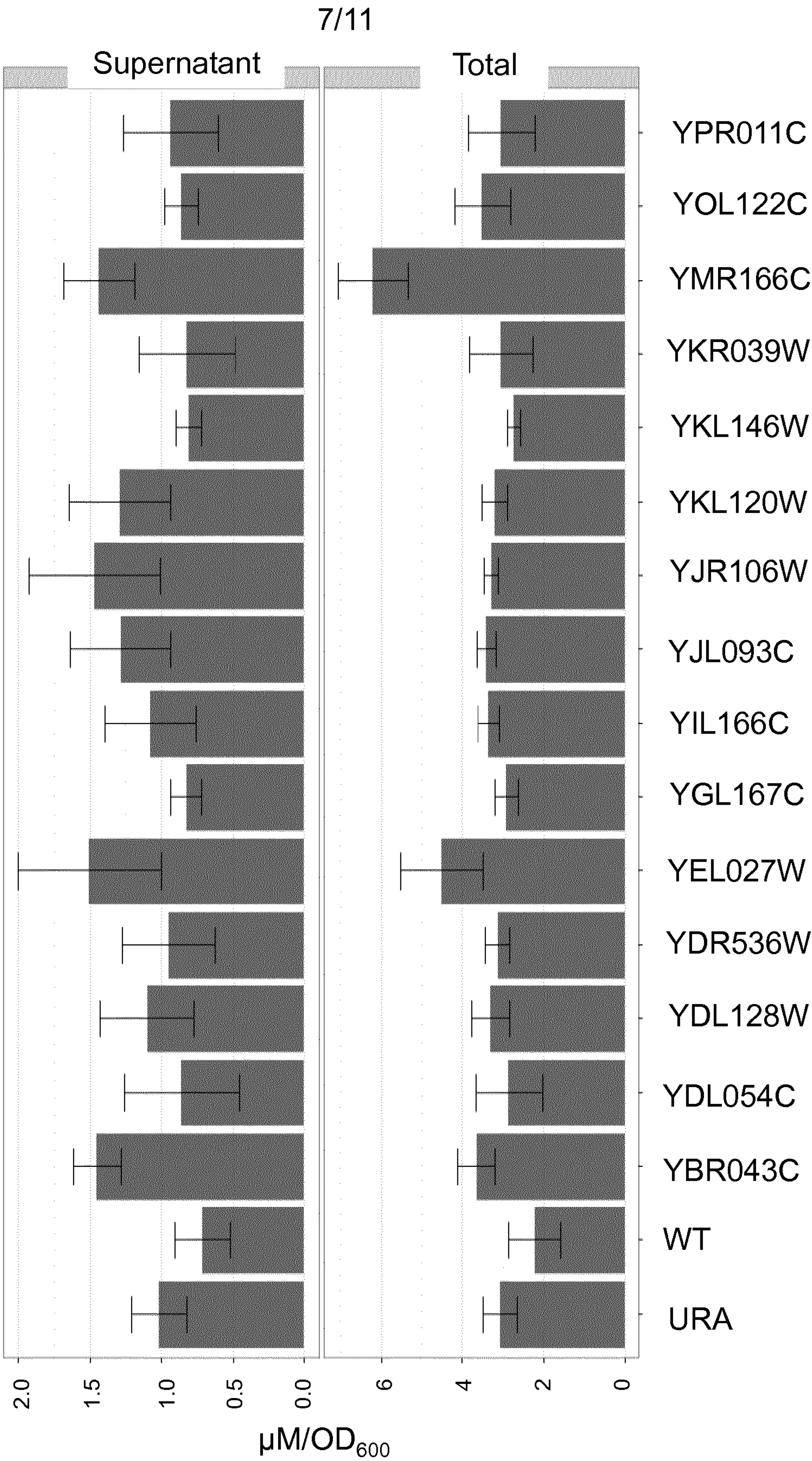


Figure 4C
RebB



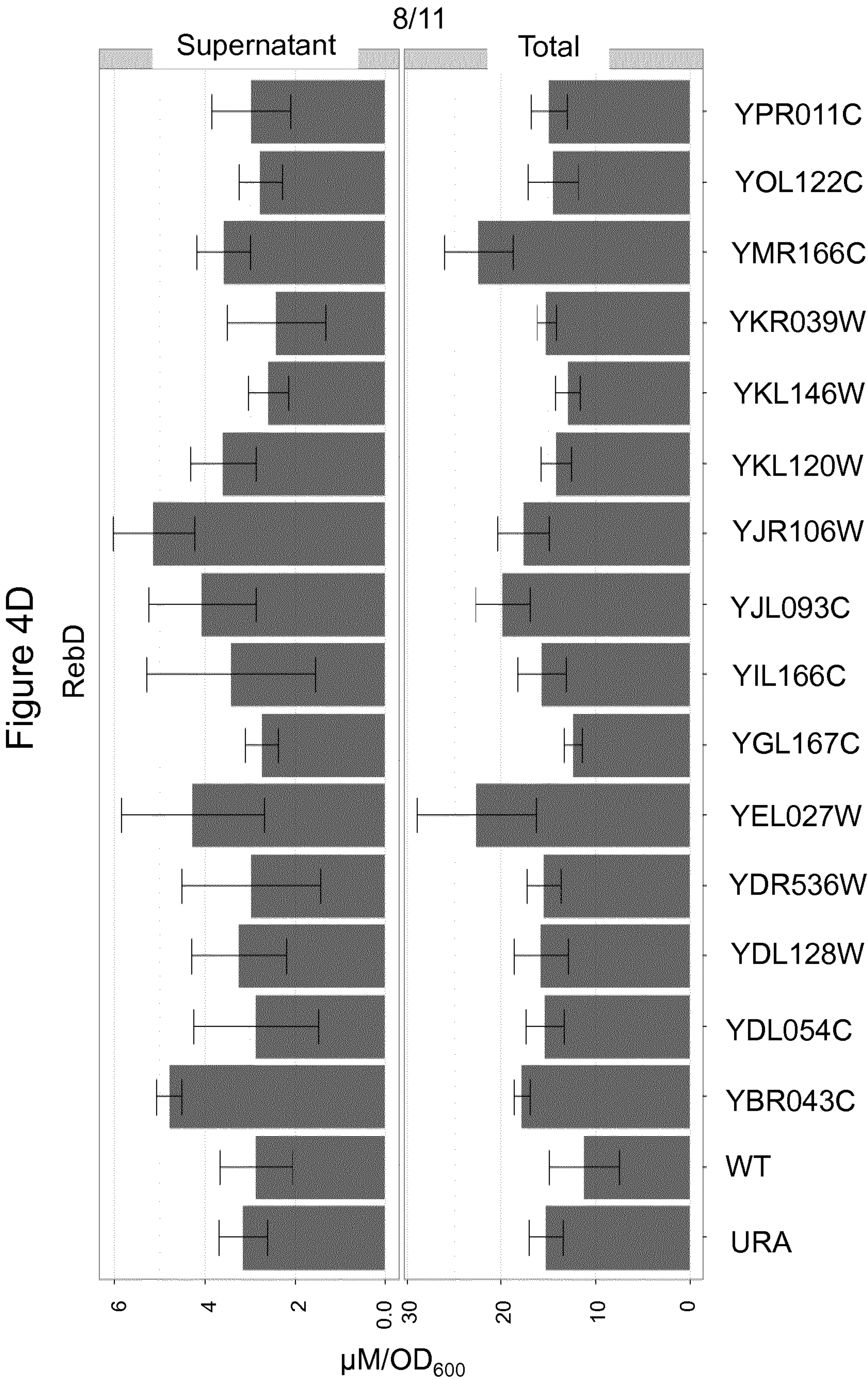
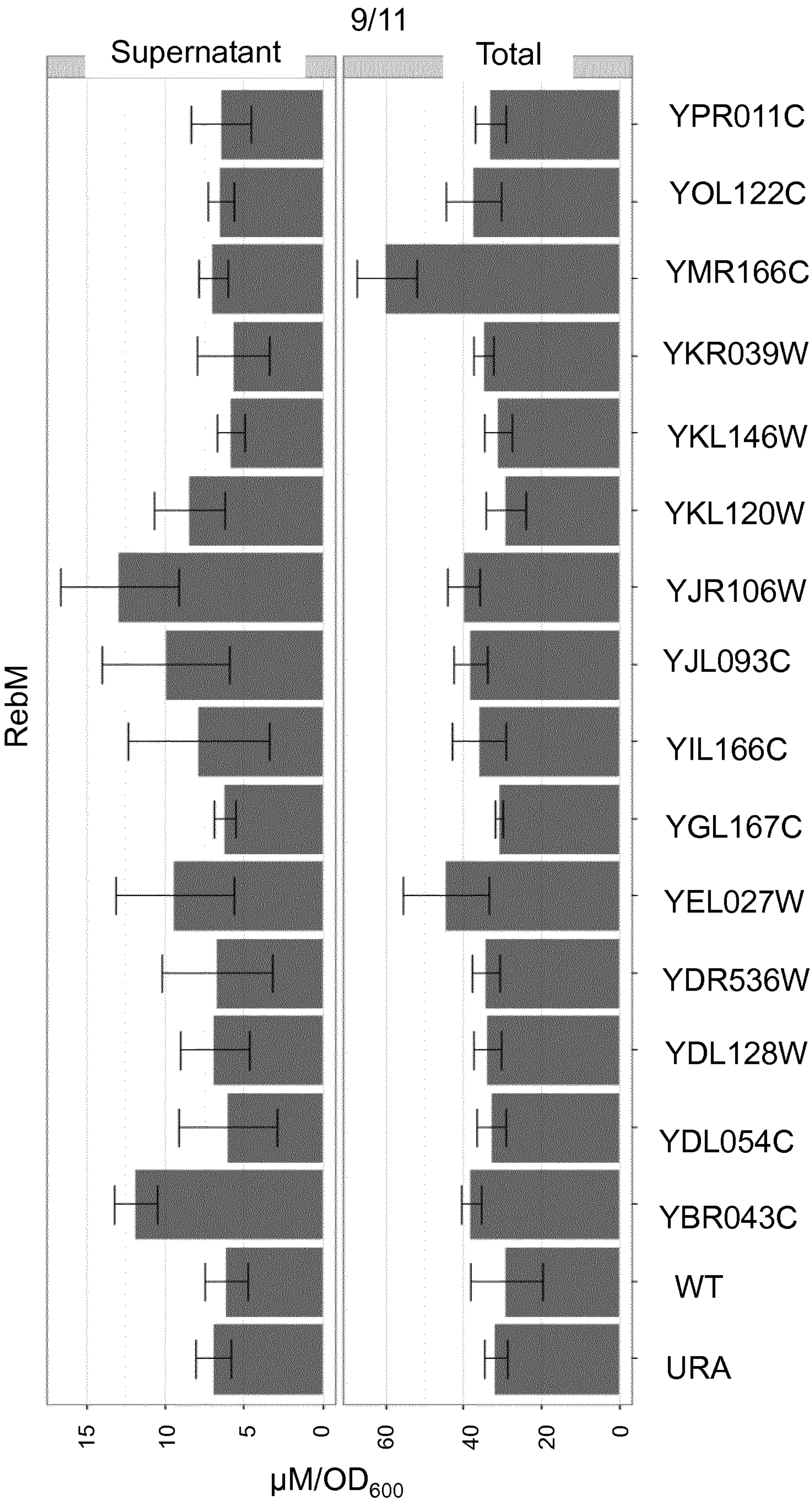


Figure 4E



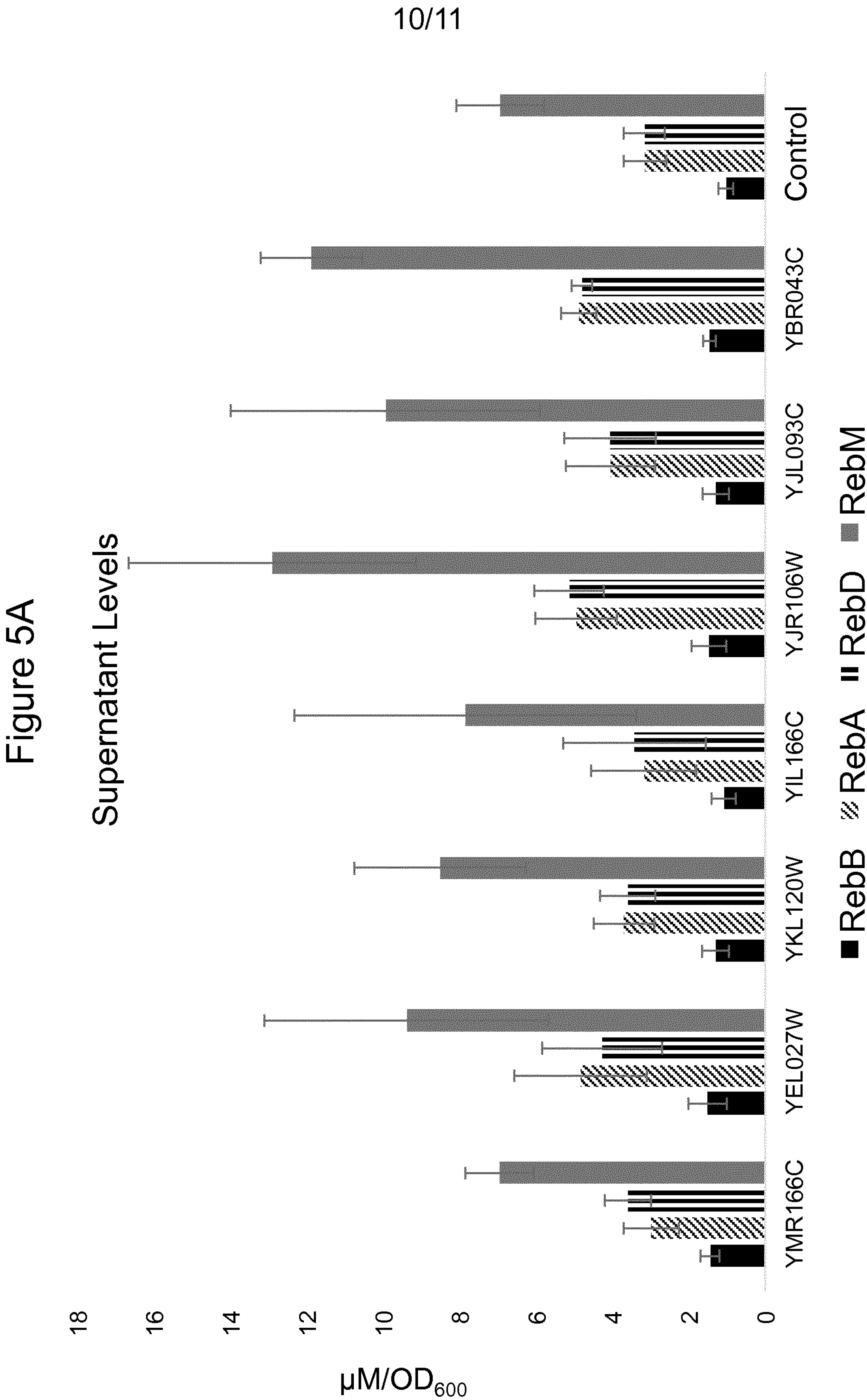


Figure 5B
Total Levels

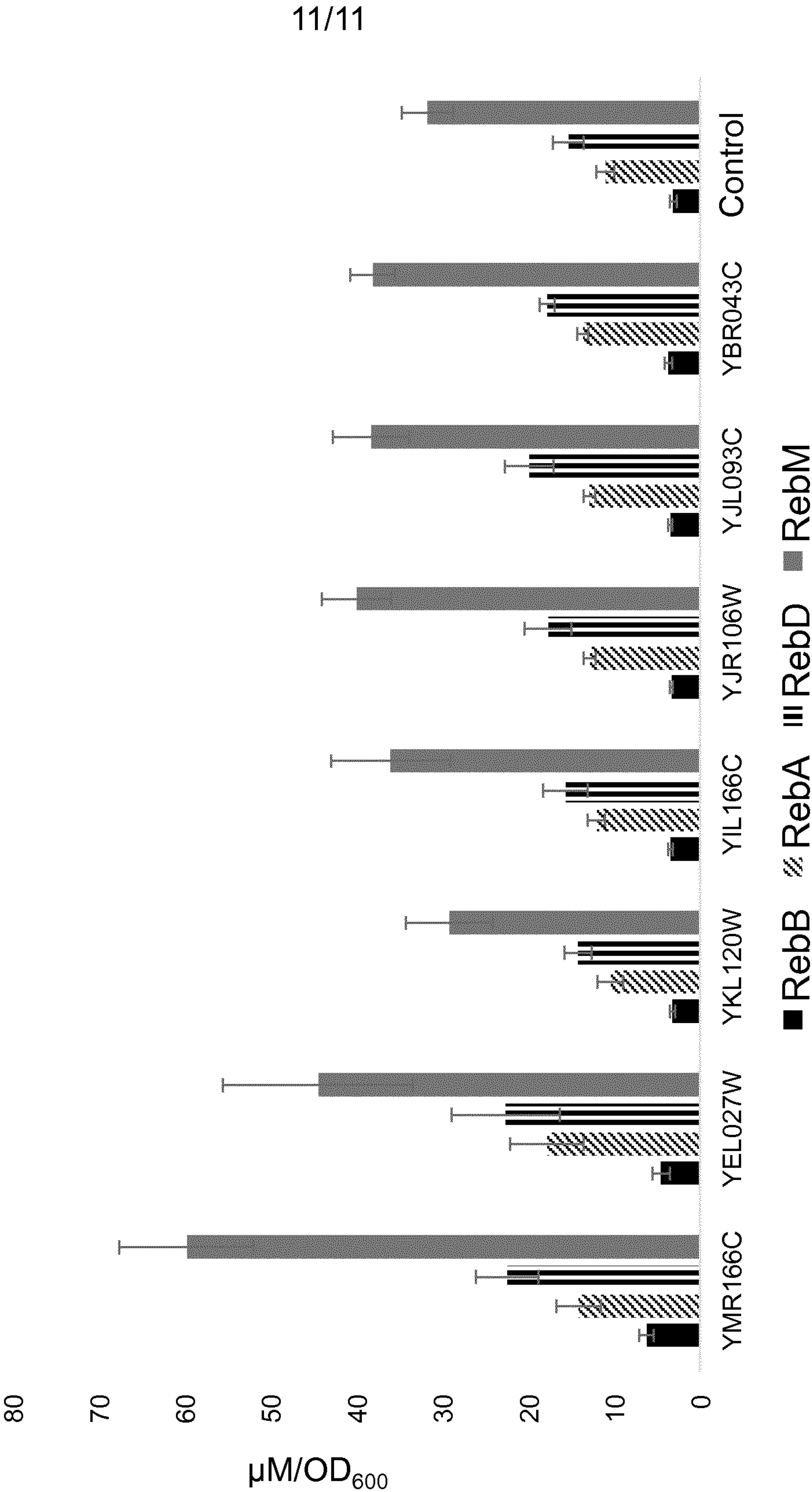


Figure 1

