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(54) Title: COMPOSITIONS AND METHODS FOR REDUCING TREHALOSE DURING THE PRODUCTION OF XYLITOL

(57) Abstract: Disclosed herein are compositions and methods for reducing trehalose during the production of xylitol by fermentation. The method comprises contacting a substrate comprising dextrose in a fermentation broth with an engineered cell capable of producing xylitol, wherein fermentation of the substrate by the engineered cell produces xylitol. During fermentation, a trehalase enzyme is present in the fermentation broth such that the concentration of trehalose at the end of fermentation is lower than an equivalent fermentation run without the trehalase enzyme.

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COMPOSITIONS AND METHODS FOR REDUCING TREHALOSE DURING THE PRODUCTION OF XYLITOL

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/594,063, filed October 30, 2023, which is incorporated by reference herein in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED VIA PATENT CENTER

[0002] The content of the Sequence Listing XML file of the sequence listing named "PT-1892-WO-PCT.xml" which is 397,770 bytes in size created on October 28, 2024 and electronically submitted via Patent Center herewith the application is incorporated by reference in its entirety.

BACKGROUND

[0003] Xylitol is a low-calorie sweetener used as a food additive and sugar substitute. Commonly used in drug, dietary supplement, confectionary, and toothpaste compositions, xylitol has also been associated with anticariogenic properties when used in chewing gums. Traditional methods of xylitol production, including chemically catalyzed hydrogenation of xylose hydrolyzed from biomass extracted xylan, are both monetarily and environmentally costly. These methods require high temperatures and pressures, large amounts of water, and metal catalysts that must be mined. In contrast, fermentation processes have been used commercially at large scale to produce other organic molecules, such as ethanol, citric acid, lactic acid, and the like, and may offer a cost effective and sustainable alternative to traditional xylitol processing methods.

[0004] In the development of microorganism-based fermentation strategies for the production of xylitol, production of metabolic pathway intermediates and alternative fermentation products are important considerations. For example, microorganism-based fermentation of xylitol may also result in the production of degree of polymerization 2 (DP2) sugars such as trehalose. However, production of trehalose may pull carbon away from the production of xylitol and there is potential to reuse the two molecules of glucose that make up trehalose to increase titer and/or yield of xylitol. Accordingly, provided herein are genetically modified yeast and fermentation methods for the production of xylitol, wherein trehalose is also reduced or eliminated from the fermentation broth during fermentation.

SUMMARY

[0005] The present disclosure provides a method for producing xylitol, the method comprising contacting a substrate comprising dextrose in a fermentation broth with an engineered cell capable of producing xylitol, wherein fermentation of the substrate by the engineered cell produces xylitol, and wherein during fermentation, a trehalase enzyme is present in the fermentation broth such that the concentration of trehalose at the end of fermentation is lower than an equivalent fermentation run without the trehalase enzyme. Trehalose titer is reduced relative to an equivalent method lacking the trehalase enzyme, e.g., trehalose titer is reduced at least 25%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or 100% relative to an equivalent method lacking the trehalase enzyme. Trehalose titer may be zero (0) or may be below the detection limit. The trehalase enzyme may be added to the broth exogenously. The engineered cell may comprise an exogenous polynucleotide sequence encoding a secretion signal operable linked to a trehalase enzyme and the trehalase enzyme may be excreted from the engineered cell. The polypeptide sequence of the secretion signal may be at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:29-32 and the polypeptide sequence of the trehalase enzyme may be at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:25-28, preferably SEQ ID NOs:25 or 26. The polynucleotide sequence encodes a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of 33, 35, 36, 38, 39, 40, and 43, preferably SEQ ID NOs:35, 36, 38, and 40, most preferably SEQ ID NOs:35, 36, and 40. The fermentation temperature may be at or between 25 °C to 45 °C, 30 °C to 40 °C, or 32 °C to 37 °C. The volumetric oxygen uptake rate (OUR) may be between 5-80, 10-75, 15-70, 20-60, 30-50, or 40-50 mmol O_2/(L • h) The xylitol may be produced at a rate of at least 0.2, 0.3, 0.5, 0.75, or at least 1.0 g L^{-1} h^{-1}. Xylitol production may be at least at least 20, 30, 50, 75, or 100 g/L when the fermentation is run at 35 °C for 96 hours. The concentration of dextrose may be at least 100 g/L.

[0006] Also provided herein is a genetically engineered yeast cell capable of producing xylitol, the engineered yeast cell comprising an exogenous polynucleotide sequence encoding a secretion signal operable linked to a trehalase enzyme and the trehalase enzyme may be excreted from the engineered cell. The polypeptide sequence of the secretion signal may be at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:29-32 and the polypeptide sequence of the trehalase enzyme may be at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:25-28, preferably SEQ ID NOs:25 or 26. The polynucleotide sequence encodes a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of 33, 35, 36, 38, 39, 40, and 43, preferably SEQ ID NOs:35, 36, 38, and 40, most preferably SEQ ID NOs:35, 36, and 40. The yeast cell may be an osmotolerant yeast cell. The yeast cell may be a cell of the subphylum Ustilaginomycotina. The yeast cell may be selected form the group consisting of *Trichosporonoides megachiliensis*, *Trychosporonoides oedocephalis*, *Trychosporonoides nigrescens*, *Pseudozyma tsukubaensis*, *Trigonopsis variabilis*, *Moniliella*, *Ustilaginomycetes*, *Trichosporon*, *Yarrowia lipolytica*, *Penicillium*, *Torula*, *Pichia*, *Candida*, *Candida magnoliae*, and *Aureobasidium*. The yeast cell may be a *Moniliella pollinis* cell.

[0007] The yeast cell may be a *Moniliella pollinis* cell and additionally comprise a deletion or disruption of a gene encoding an erythrose reductase enzyme at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:74 and 76. The cell may have a deletion of at least one allele of the gene encoding the erythrose reductase enzyme. The cell may have a deletion of both alleles of the gene encoding the erythrose reductase enzyme.

[0008] The cell may additionally comprise an exogenous polynucleotide sequence encoding a xylitol-phosphate dehydrogenase (XPDH) enzyme comprising a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs: 5, 108-114, and 116. The XPDH enzyme may have a sequence at least 85% identical to at least one of SEQ ID NOs: 5, 108-114, and 116 or to at least one of SEQ ID NOs:5, 110, 111, and 114. The XPDH enzyme has a sequence at least 90% identical to at least one of SEQ ID NOs: 5, 108-114, and 116 or to at least one of SEQ ID NOs:5, 110, 111, and 114.

[0009] The cell may additionally comprise an exogenous polynucleotide sequence encoding a xylulokinase (XKS) enzyme comprising a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:103 and 104. The cell may additionally comprise an exogenous polynucleotide sequence encoding a xylitol dehydrogenase (XDH) enzyme comprising a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:117-119.

[0010] The cell may additionally comprise a genetic modification resulting in overexpression of a native enzyme with ribulose-5-phosphate epimerase (RPE) activity. The yeast cell may be a yeast cell of the genus *Moniliella*. The yeast cell may be a *Moniliella pollinis* cell and the native RPE enzyme comprises a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:14 and 107. The genetic modification resulting in overexpression of a native RPE enzyme comprises addition of an exogenous polynucleotide encoding the native RPE enzyme such that the genetically engineered cell comprises at least one additional copy of a sequence encoding the RPE enzyme.

[0011] The yeast may additionally comprise a genetic modification resulting in overexpression of a native enzyme with xylitol-5-phosphate phosphatase (X5PP) activity; and/or an exogenous polynucleotide sequence encoding an enzyme with xylitol-5-phosphate phosphatase (X5PP) activity. The cell may be a *Moniliella pollinis* cell and the genetic modification results in overexpression of a native X5PP enzyme with a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:24, 101, 105, or 106. The genetic modification comprises addition of an exogenous polynucleotide sequence encoding the native X5PP enzyme such that the genetically engineered cell comprises at least one additional copy of a sequence encoding the native X5PP enzyme. The yeast cell may comprise an exogenous polynucleotide sequence encoding an enzyme with X5PP activity and a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs: 24 and 88-104. The yeast cell comprises an exogenous polynucleotide sequence encoding an enzyme with X5PP activity and a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs: 24, 89-93, 97, and 99. The yeast cell comprises an exogenous polynucleotide sequence encoding an enzyme with X5PP activity and a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs: 24, 90, 91, 93, and 99. X5PP activity in the genetically engineered yeast cell may be higher than X5PP activity in an equivalent cell lacking the genetic modification or exogenous polynucleotide sequence.

[0012] One or more of the exogenous polynucleotide sequences described above may be operably linked to a heterologous or artificial promoter. The heterologous or artificial promoter may be selected from the group consisting of pyruvate kinase 1 promoter (PYK1p; SEQ ID NO:6),

6-phosphogluconate dehydrogenase promoter (6PGDp; SEQ ID NO:120), glyceraldehyde-3-phosphate dehydrogenase promoter (TDH3p; SEQ ID NO:121), translational elongation factor 1 promoter (TEFp; SEQ ID NO:122), phosphoglucomutase 1 promoter (PGM1p; SEQ ID NO:123), 3-phosphoglycerate kinase promoter (PGK1p; SEQ ID NO:22), enolase promoter (ENO1p ; SEQ ID NO:124), asparagine synthetase promoter (ASNSp; SEQ ID NO:125), 50S ribosomal protein L1 promoter (RPLAp; SEQ ID NO:126), and RPL16B (SEQ ID NO:127). The promoter may be a constitutive promoter. One or more of the exogenous polynucleotide sequences is integrated into the genome of the yeast cell at a locus selected from the ER1 locus, the ER3 locus, the PDC1 locus, the pyrF locus, the TRP3 locus, the gpdIIA locus, and the gpdIIB locus.

[0013] The disclosure also provides a method for producing xylitol using the engineered cells described herein, the method comprising contacting a substrate comprising dextrose with an engineered cell described herein, wherein fermentation of the substrate by the engineered cell produces xylitol. The fermentation temperature may be at or between 25 °C to 45 °C, 30 °C to 40 °C, or 32 °C to 37 °C. The volumetric oxygen uptake rate (OUR) may be between 5-80, 10-75, 15-70, 20-60, 30-50, or 40-50 mmol O_2/(L • h) The xylitol may be produced at a rate of at least 0.2, 0.3, 0.5, 0.75, or at least 1.0 g L^{-1} h^{-1}. Xylitol production may be at least at least 20, 30, 50, 75, or 100 g/L when the fermentation is run at 35 °C for 96 hours. The concentration of dextrose may be at least 100 g/L.

[0014] The disclosure also provides use of the engineered cells described herein to produce xylitol in a fermentation process with reduced trehalose.

BRIEF DESCRIPTION OF THE FIGURES

[0015] This patent or application contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and the payment of the necessary fee.

[0016] The drawings illustrate generally, by way of example, but not by way of limitation, various aspects discussed herein.

[0017] FIG. 1 shows the native pentose phosphate pathway (dotted lines and arrows) and the native glycolysis pathways (solid lines and arrows) in *Moniliella pollinis*.

[0018] FIG. 2 shows deep well plate xylitol and trehalose titer (g/L) results as described in Example 2.

[0019] FIG. 3 shows erythritol and xylitol titer (g/L) results as described in Example 3.

[0020] FIG. 4 shows trehalose titer (g/L) results as described in Example 3.

[0021] FIG. 5 shows xylitol yield (%) at 72 and 96 hours as described in Example 3. Black horizontal lines show the xylitol yield of the control strain 8-1 for comparison.

DETAILED DESCRIPTION

[0022] Reference will now be made in detail to certain aspects of the disclosed subject matter, examples of which are illustrated in part in the accompanying drawings. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not intended to limit the claims to the disclosed subject matter.

[0023] In this document, the terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. All publications, patents, and patent documents referred to in this document are incorporated by reference herein in their entirety, as though individually incorporated by reference. In the event of inconsistent usages between this document and those documents so incorporated by reference, the usage in the incorporated reference should be considered supplementary to that of this document; for irreconcilable inconsistencies, the usage in this document controls.

[0024] Values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range were explicitly recited. For example, a range of "about 0.1% to about 5%" or "about 0.1% to 5%" should be interpreted to include not just about 0.1% to about 5%, but also the individual values (e.g., 1%, 2%, 3%, and 4%) and the sub-ranges (e.g., 0.1% to 0.5%, 1.1% to 2.2%, 3.3% to 4.4%) within the indicated range. The statement "about X to Y" has the same meaning as "about X to about Y," unless indicated otherwise. Likewise, the statement "about X, Y, or about Z" has the same meaning as "about X, about Y, or about Z," unless indicated otherwise.

[0025] Unless expressly stated, ppm (parts per million), percentage, and ratios are on a by weight basis. Percentage on a by weight basis is also referred to as wt% or % (wt) below.

[0026] This disclosure relates to compositions and methods for reducing trehalose in the fermentation broth during the production of xylitol. The disclosure includes various recombinant cells engineered to produce and excrete a trehalase enzyme. In general, the recombinant cells

described herein are capable of producing xylitol and comprise an exogenous polynucleotide sequence encoding a trehalase enzyme. The recombinant yeast may additionally be characterized by overexpression of a ribulose 5-phosphatase epimerase (RPE) enzyme; inclusion of an exogenous polynucleotide sequence encoding a xylitol phosphate dehydrogenase (XPDH) enzyme; inclusion of an exogenous polynucleotide sequence encoding a xylulokinase (XKS) enzyme; inclusion of an exogenous polynucleotide sequence encoding a xylitol dehydrogenase (XDH) enzyme; and/or overexpression of a xylitol-5-phosphate phosphatase (X5PP) enzyme. The disclosure also includes compositions and methods in which the trehalase enzymes is added to the fermentation broth directly. The disclosure further provides fermentation methods for the production of xylitol from dextrose using the genetically engineered cells described herein.

[0027] In general, recombinant cells described herein are yeast cells. As used herein, "yeast" refers to eukaryotic single celled microorganisms classified as members of the fungus kingdom. Yeast are unicellular organisms which evolved from multicellular ancestors with some species retaining multicellular characteristics such as forming strings of connected budding cells known as pseudo hyphae or false hyphae. Yeast cells may also be referred to in the art as yeast-like cells, and as used herein "yeast cell" encompasses both yeast and yeast-like cells. Suitable yeast and yeast-like host cells for modification may include, but are not limited to, *Saccharomyces cerevisiae*, *Komagataella sp.*, *Kluyveromyces* (e.g., *Kluyveromyces lactis, Kluveromyces marxianus*), *Yarrowia lipolytica*, *Issatchenkia orientalis*, *Pichia galeiformis*, *Pichia sp.* YB-4149 (NRRL designation), *Pichia pastoris*, *Candida* (e.g., *Candida magnoliae*, *Candida ethanolica*), *Pichia deserticola*, *Pichia membranifadens*, *Pichia fermentans*, *Aspergillus*, *Trichoderma*, *Myceliphthora thermophila, Moniliella* (e.g., *Moniliella pollinis*), *Pfaffia, Yamadazyma, Hansenula, Pichia kudriavzevvi, Trichosporonoides* (e.g.,*Trichosporonoides megachiliensis, Trychosporonoides oedocephalis, Trychosporonoides nigrescens*), *Pseudozyma tsukubaensis, Trigonopsis variabilis, Penicillium,* and *Torula*. An ordinarily skilled artisan would understand the requirements for selection of a suitable yeast cell, and recombinant yeast cells of the present disclosure are not limited to those expressly recited herein. Methods for genetic engineering of yeast cells are known and described in the art and a skilled artisan would understand the methods necessary to transform and engineer a suitable yeast cell.

[0028] A suitable yeast cell may be a cell of the phylum *Basidiomycota* and the subphylum *Ustilaginomycotina*. Suitable yeast of the subphylum *Ustilaginomycotina* include, but are not limited to, *Ustilago* (e.g., *U. cynodontis*, *U. maydis*, *U. sphaerogena, U. cordal, U. scitaminea, U.*

coicis, U. syntherismae, U. esculenta, U. neglecta, U. crus-galli, *Ustilago avenae*), *Sporisorium* (e.g., *Sporisorium exsertum*), *Moniliella* (e.g., *M. pollinis, M. tomentosa, M. acetoabutans, M. fonsecae, M. madida, M. megachiliensis, M. ocedocephalis, M. nigrescens*), and *Pseudozyma* (e.g., *Pseudozyma tsukubaensis*), and *Trichosporonoides* (e.g., *Trichosporonoides megachiliensis, Trychosporonoides oedocephalis, Trychosporonoides nigrescens*). Yeast of the subphylum *Ustilaginomycotina* have been known and described in the art as potential production organisms for valuable chemicals such as itaconate, malate, succinate, mannitol, and erythritol and other valuable biotechnological applications. See, for example, Geiser et al. (Prospecting the biodiversity of the fungal family Ustilaginacceae for the production of value-added chemicals," Fungal Biol Biotechnol, 2014, 1:2), Feldbrugge et al., ("The biotechnological use and potential of plant pathogenic smut fungi," Appl Microbiol Biotechnol, 2013, 97(8):3253-65), Guevarra et al., ("Accumulation of itaconic, 2-hydroxyparaconic, itatartaric, and malic acids by strains of the genus Ustilago, Agric. Biol. Chem., 1990, 54(9), 2353-2358), and Moon et al., ("Biotechnological production of erythritol and its applications," Appl Microbiol Biotechnol, 2010, 86:1017-1025).

[0029] A suitable yeast cell will have an active pentose phosphate pathway that produces ribulose-5-phosphate. As used herein "active pentose phosphate pathway" refers to expression of one or more functional enzymes which, together, convert glucose-6-phosphate, $NADP^+$ or NAD+ ($NAD(P)^+$), and water to NADPH or NADH (NAD(P)H), CO2, and ribulose-5-phosphate. Continuing in a non-oxidative phase, the pathway may also produce other pentose (i.e., 5-carbon) sugars. For example, the pentose phosphate pathway may produce ribulose-5-phosphate, ribose-5-phosphate, xylulose-5-phosphate, fructose 6-phosphate, combinations thereof, and the like, depending on the enzymatic activities present. The active pentose phosphate pathway may be native to the yeast cell, or it may be introduced into the yeast cell by genetic engineering.

[0030] The yeast cell may be an osmotolerant yeast cell. As used herein, "osmotolerant" refers to a yeast capable of growth and reproduction under conditions of high osmolarity, such as at least 10% (w/v), at least 20% (w/v), at least 30% (w/v), at least 40% (w/v), at least 50% (w/v), or at least 60% (w/v) glucose and/or at least 6% (w/v), at least 10% (w/v), at least 12% (w/v), at least 13% (w/v), at least 15% (w/v) sodium chloride. Species and strains of osmotolerant yeast are known and described in the art, including many species of yeast used in industrial fermentation processes. Likewise, methods for assaying yeast osmotolerance are known and described in the art. See, for example, Tiwari, S., et al., ("Nectar yeast community of tropical flowering plants and

assessment of their osmotolerance and xylitol-producing potential," Current Microbiology, 2022, 79:28).

[0031] The recombinant yeast cell may be a recombinant *Moniliella* cell, for example, a *Moniliella pollinis* cell. FIG. 1 shows the predicted native pentose phosphate and glycolysis pathways in *Moniliella pollinis*. *Moniliella* has previously been used in the fermentation production of erythritol and methods for genetically modifying and fermenting *Moniliella* are known and described in the art. See, for example, Li et al. ("Methods for genetic transformation of filamentous fungi," 2017, Microb Cell Fact, 16:168).

[0032] Various plasmids and methods for transformation of *Moniliella* are also described in the Examples below. For example, *Moniliella* may be transformed using a bipartite polynucleotide sequence in which, following recombination, the exogenous polynucleotide of interest is integrated at the specified locus and the selection marker is expressible within the cell. Suitable selection markers are known and used in the art. The selectable marker may include, but is not limited to, amdS (for example broken into a 3' portion, SEQ ID NO:68, and a 5' portion, SEQ ID NO:69), G418 resistance gene (for example broken into a 3' portion, SEQ ID NO:9, and a 5' portion, SEQ ID NO:8), zeocin resistance gene (for example broken into a 3' portion, SEQ ID NO:17, and a 5' portion, SEQ ID NO:16), nourseothricin N-acetyl transferase (NAT) (for example broken into a 3' portion, SEQ ID NO:70, and a 5' portion, SEQ ID NO:71), and invertase gene (SUC2) (for example a 3' portion of SEQ ID NO:72 and a 5' portion of SEQ ID NO:73).

[0033] The recombinant cells described herein include one or more exogenous polynucleotide sequences encoding one or more polypeptides that, when expressed, improve the fermentation of glucose to xylitol by the recombinant cells.

[0034] The terms "glucose" and "dextrose" are used interchangeably herein and refer to D-glucose except where expressly indicated otherwise.

[0035] As used herein, "exogenous" refers to genetic material or an expression product thereof that originates from outside of the host organism. For example, the exogenous genetic material or expression product thereof can be a modified form of genetic material native to the host organism, it can be derived from another organism, it can be a modified form of a component derived from another organism, or it can be a synthetically derived component. For example, a *K. lactis* invertase gene is exogenous when introduced into *S. cerevisiae*.

[0036] As used herein, "native" refers to genetic material or an expression product thereof that is found, apart from individual-to-individual mutations which do not affect function or expression,

within the genome of wild-type cells of the host cell. For the purposes of this application, the *Moniliella pollinis* cell "*Moniliella tomentosa* var *pollinis* TCV364" described in US 6,440,712, which is incorporated herein by reference in its entirety, and deposited under the Budapest Treaty at BCCM/MUCL (Belgian Coordinated Collections of Micro-organisms/Mycothèque de l'Université Catholique de Louvain by Eridania Béghin Say, Vilvoorde R&D Centre, Havenstraat 84, B-1800 Vilvoorde) on March 28, 1997 under number MUCL40385, is considered the wild-type *Moniliella pollinis* cell.

[0037] As used herein, the terms "polypeptide" and "peptide" are used interchangeably and refer to the collective primary, secondary, tertiary, and quaternary amino acid sequences and structure necessary to give the recited macromolecule its function and properties. As used herein, "enzyme" or "biosynthetic pathway enzyme" refer to a protein that catalyzes a chemical reaction. The recitation of any particular enzyme, either independently or as part of a biosynthetic pathway is understood to include the co-factors, co-enzymes, and metals necessary for the enzyme to properly function. A summary of the amino acids and their three and one letter symbols as understood in the art is presented in Table 1. The amino acid name, three letter symbol, and one letter symbol are used interchangeably herein.

Table 1: Amino Acid three and one letter symbols

Amino Acid	Three-letter symbol	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M

Amino Acid	Three-letter symbol	One-letter symbol
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0038] Variants or sequences having substantial identity or homology with the polypeptides described herein can be utilized in the practice of the disclosed recombinant cells, compositions, and methods. Such sequences can be referred to as variants or modified sequences. That is, a polypeptide sequence can be modified yet still retain the ability to exhibit the desired activity. Generally, the variant or modified sequence may include greater than about 45%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sequence identity with the wild-type, naturally occurring polypeptide sequence, or with a variant polypeptide as described herein.

[0039] As used herein, the phrases "% sequence identity," "% identity," and "percent identity," are used interchangeably and refer to the percentage of residue matches between at least two amino acid sequences or at least two nucleic acid sequences aligned using a standardized algorithm. Methods of amino acid and nucleic acid sequence alignment are well-known. Sequence alignment and generation of sequence identity include global alignments and local alignments which are carried out using computational approaches. An alignment can be performed using BLAST (National Center for Biological Information (NCBI) Basic Local Alignment Search Tool) version 2.2.31 software with default parameters. Amino acid % sequence identity between amino acid sequences can be determined using standard protein BLAST with the following default parameters: Max target sequences: 100; Short queries: Automatically adjust parameters for short input sequences; Expect threshold: 10; Word size: 6; Max matches in a query range: 0; Matrix: BLOSUM62; Gap Costs: (Existence: 11, Extension: 1); Compositional adjustments: Conditional compositional score matrix adjustment; Filter: none selected; Mask: none selected. Nucleic acid % sequence identity between nucleic acid sequences can be determined using standard nucleotide BLAST with the following default parameters: Max target sequences: 100; Short queries: Automatically adjust parameters for short input sequences; Expect threshold: 10; Word size: 28;

Max matches in a query range: 0; Match/Mismatch Scores: 1, -2; Gap costs: Linear; Filter: Low complexity regions; Mask: Mask for lookup table only. A sequence having an identity score of XX% (for example, 80%) with regard to a reference sequence using the NCBI BLAST version 2.2.31 algorithm with default parameters is considered to be at least XX% identical or, equivalently, have XX% sequence identity to the reference sequence.

[0040] Polypeptide or polynucleotide sequence identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0041] The polypeptides disclosed herein may include "variant" polypeptides, "mutants," and "derivatives thereof." As used herein the term "wild-type" is a term of the art understood by skilled persons and means the typical form of a polypeptide as it occurs in nature as distinguished from variant or mutant forms. As used herein, a "variant," "mutant," or "derivative" refers to a polypeptide molecule having an amino acid sequence that differs from a reference protein or polypeptide molecule. A variant or mutant may have one or more insertions, deletions, or substitutions of an amino acid residue relative to a reference molecule.

[0042] The amino acid sequences of the polypeptide variants, mutants, derivatives, or fragments as contemplated herein may include conservative amino acid substitutions relative to a reference amino acid sequence. For example, a variant, mutant, derivative, or fragment polypeptide may include conservative amino acid substitutions relative to a reference molecule. "Conservative amino acid substitutions" are those substitutions that are a substitution of an amino acid for a different amino acid where the substitution is predicted to interfere least with the properties of the reference polypeptide. In other words, conservative amino acid substitutions substantially conserve the structure and the function of the reference polypeptide. Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge and/or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

[0043] As used herein, terms "polynucleotide," "polynucleotide sequence," and "nucleic acid sequence," and "nucleic acid," are used interchangeably and refer to a sequence of nucleotides or any fragment thereof. These phrases also refer to DNA or RNA of natural or synthetic origin, which may be single-stranded or double-stranded and may represent the sense or the antisense strand. The DNA polynucleotides may be a cDNA (e.g., coding DNA) or a genomic DNA sequence (e.g., including both introns and exons).

[0044] A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

[0045] Those of skill in the art understand the degeneracy of the genetic code and that a variety of polynucleotides can encode the same polypeptide. In some aspects, the polynucleotides (e.g., polynucleotides encoding an erythrose reductase polypeptide) may be codon-optimized for expression in a particular cell including, without limitation, a plant cell, bacterial cell, fungal cell, or animal cell. While polypeptides encoded by polynucleotide sequences found in various species are disclosed herein any polynucleotide sequences may be used which encodes a desired form of the polypeptides described herein. Thus, non-naturally occurring sequences may be used. These may be desirable, for example, to enhance expression in heterologous expression systems of polypeptides or proteins. Computer programs for generating degenerate coding sequences are available and can be used for this purpose. Pencil, paper, the genetic code, and a human hand can also be used to generate degenerate coding sequences.

[0046] The recombinant cells described herein may include deletions or disruptions in one or more native genes. The phase "deletion or disruption" refers to the status of a native gene in the recombinant cell that has either a completely eliminated coding region (deletion) or a modification of the gene, its promoter, or its terminator (such as by a deletion, insertion, or mutation) so that the gene no longer produces an active expression product, produces severely reduced quantities of the expression product (e.g., at least a 75% reduction or at least a 90% reduction) or produces an expression product with severely reduced activity (e.g., at least 75% reduced or at least 90% reduced). The deletion or disruption can be achieved by genetic engineering methods, forced evolution, mutagenesis, RNA interference (RNAi), and/or selection and screening. Deletion or disruption of a native host cell gene can be coupled to the incorporation of one or more polynucleotide sequences (e.g., an exogenous or native polynucleotide sequence) into the host cell

at the locus of the host cell gene to be deleted or disrupted. The polynucleotide sequence to be inserted may be designed to replace all or a portion of the host cell gene to be deleted or disrupted. The polynucleotide sequence may encode for a gene product of interest, for example, a polypeptide, an enzyme, and the like. The deletion or disruption can also be accomplished using a deletion construct that does not contain a polynucleotide sequence to be integrated. Other methods for gene disruption or deletion are known and described in the art.

[0047] The recombinant cells described herein have a deletion or disruption in one or more native genes encoding an enzyme involved in erythritol fermentation or consumption. Deletion or disruption of one or more of these biosynthetic pathway enzymes decreases the ability of the recombinant cell to produce erythritol and may, depending on the deletion or disruption, increasing carbon flux into the fermentation pathway for the production of xylitol.

[0048] The recombinant cells described herein may include a deletion or disruption of a native erythrose reductase (ER) gene. The native ER gene encodes an enzyme that has erythrose reductase activity. As used herein "erythrose reductase activity" and "ER activity" are used interchangeably and refer to enzymes that catalyze the reversible conversion of erythrose or erythrose-4-phosphate to erythritol or erythritol-4-phosphate using a nicotinamide adenine dinucleotide (phosphate) (hydrogen) (NAD(P)(H)) cofactor. In the art, enzymes that catalyze the reversible conversion of erythrose-4-phosphate to erythritol-4-phosphate using a nicotinamide adenine dinucleotide (phosphate) (hydrogen) (NAD(P)(H)) cofactor may also be described as erythrose or erythritol phosphate dehydrogenases. When the host cell contains multiple ER genes, it is preferred to delete or disrupt at least one of them. When the host cell contains multiple alleles of a given ER gene, it is preferred to delete or disrupt one allele or both alleles of the given ER gene.

[0049] As used herein, "NAD(P)H" refers to nicotinamide adenine dinucleotide (phosphate) hydrogen and is inclusive of both NADH and NADPH. As is understood in the art, inclusion of the phosphate (or "P" abbreviation) in parentheses indicates that the phosphate may be absent or present and the name and abbreviation are inclusive of both. Similarly, "NAD(H)" or "NADP(H)" refers to both the reduced and oxidized forms of the cofactor.

[0050] When the recombinant cell is a *Moniliella pollinis* cell, the recombinant cell may comprise a deletion or disruption of an ER gene encoding an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:74. When the recombinant cell is a *Moniliella pollinis* cell, the recombinant cell may comprise a deletion or disruption of an ER gene with a

nucleotide sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:75. See, for example, US Provisional Application No. 63/499,990, filed May 4, 2023, and US Provisional Application No. 63/499,989, filed May 4, 2023.

[0051] When the recombinant cell is a *Moniliella pollinis* cell, the recombinant cell may comprise a deletion or disruption of an ER gene encoding an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:76. When the recombinant cell is a *Moniliella pollinis cell*, the recombinant cell may comprise a deletion or disruption of an ER gene with a nucleotide sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:77. See, for example, US Provisional Application No. 63/499,990, filed May 4, 2023, and US Provisional Application No. 63/499,989, filed May 4, 2023.

[0052] The recombinant cells described herein may include one or more genetic modifications in which an exogenous nucleic acid is integrated into the genome of the host cell. One of skill in the art know how to select suitable loci in a yeast genome for integration of the exogenous nucleic acid. Suitable integration loci may include, but are not limited to, the PDC1, GPD1, CYB2A, CYB2B, g4240, YMR226, MDHB, ATO2, Adh9091, Adh1202, ADE2, ADH2556, GAL6, MDH1, SCW11, ER1, ER3, pyrF, TRP3, gpdIIA, and gpdIIB loci. For example, in a *M. pollinis* host cells, suitable interaction loci may include, but are not limited to, the *ER1* locus (defined as the locus flanked by SEQ ID NO:3 and SEQ ID NO:10), the *ER3* locus (defined as the locus flanked by SEQ ID NO:13 and SEQ ID NO:19), the *PDC1* locus (defined as the locus flanked by SEQ ID NO:78 and SEQ ID NO:79), the *pyrF* locus (defined as the locus flanked by SEQ ID NO:80 and SEQ ID NO:81), the *TRP3* locus (defined as the locus flanked by SEQ ID NO:82 and SEQ ID NO:83), the *gpdIIA* locus (defined as the locus flanked by SEQ ID NO:84 and SEQ ID NO:85); and the *gpdIIB* locus (defined as the locus flanked by SEQ ID NO:86 and SEQ ID NO:87). The exogenous nucleic acid may also be integrated in an intergenic region or other location in the host cell genome not specifically specified herein. Other suitable integration loci may be determined by one of skill in the art. Furthermore, one of skill in the art would recognize how to use sequences to design primers to verify correct gene integration at the chosen locus.

[0053] The recombinant cell may have one or more copies of a given exogenous nucleic acid sequence integrated in a host chromosome(s) and replicated together with the chromosome(s) into

which it has been integrated. For example, the yeast cell may be transformed with nucleic acid construct including a polynucleotide sequence encoding for a polypeptide described herein and the polynucleotide sequence encoding for the polypeptide may be integrated in one or more copies in a host chromosome(s). The recombinant cell may include multiple copies (two or more) of a given polynucleotide sequence encoding a polypeptide described herein. The recombinant cell may have one, two, three, four, five, six, seven, eight, nine, ten, or more copies of a polynucleotide sequence encoding a polypeptide described herein integrated into the genome. The multiple copies of said polynucleotide sequence may all be incorporated at a single locus or may be incorporated at multiple loci.

[0054] The recombinant cells described herein are capable of producing xylitol and may include an exogenous polynucleotide sequence encoding an enzyme with trehalase activity. The enzyme may be any suitable enzyme with trehalase activity. As used herein, "trehalase" and "trehalase enzyme" are used interchangeably and refer to an enzyme with trehalase activity. Herein, "trehalase activity" refers to the ability to catalyze the hydrolysis of one molecule of trehalose into two molecules of glucose. In the art trehalase enzymes may also be referred to as α,α-trehalose-1-C-glucohydrolase. Suitable enzymes with trehalase activity may include, but are not limited to, those classified under EC 3.2.1.28. Polynucleotides encoding trehalase enzymes may be derived from any suitable source. For example, a polynucleotide encoding a trehalase enzyme may be derived from *Moniliella pollinis*, *Magnaporthe grisea*, *Candida glabrata*, and the like. The trehalase enzyme may be a polypeptide with an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:25 and 26. The trehalase enzyme may be linked to a secretion signal at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:29, 30, 31, and 32. The trehalase may be linked to a secretion signal such that the recombinant cell includes an exogenous polynucleotide sequence encoding a polypeptide with an amino acid sequence at least at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:33, 35, 36, 38, 39, 40, and 43. The trehalase may be linked to a secretion signal such that the recombinant cell includes an exogenous polynucleotide sequence encoding a polypeptide with an amino acid sequence at least at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:35, 36, 38, and 40. The trehalase may be linked to a

secretion signal such that the recombinant cell includes an exogenous polynucleotide sequence encoding a polypeptide with an amino acid sequence at least at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:35, 36, and 40.

[0055] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence that is or may be derived from *M. grisea* encoding the amino acid sequence of SEQ ID NO:25. The exogenous polynucleotide sequence may encode an amino acid comprising a sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO:25. The exogenous polynucleotide sequence may encode an amino acid comprising a sequence at least 90% identical to SEQ ID NO:25.

[0056] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence that is or may be derived from *C. glabrata* encoding the amino acid sequence of SEQ ID NO:26. The exogenous polynucleotide sequence may encode an amino acid comprising a sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO:26. The exogenous polynucleotide sequence may encode an amino acid comprising a sequence at least 90% identical to SEQ ID NO:26.

[0057] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:33. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:33.

[0058] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:35. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:35.

[0059] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:36. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:36.

[0060] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:38. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:38.

[0061] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:39. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:39.

[0062] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:40. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:40.

[0063] The recombinant cell capable of producing xylitol may include an exogenous polynucleotide sequence encoding an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:43. The exogenous polynucleotide sequence may encode an amino acid sequence at least 90% identical to SEQ ID NO:43.

[0064] The recombinant cells described herein may additionally include one or more enzymes in the xylitol biosynthetic pathway. For example, the recombinant cells may include one or more of (i) a genetic modification resulting in overexpression of a native X5PP enzyme, (ii) an exogenous polynucleotide sequence encoding an exogenous X5PP enzyme, (iii) a genetic modification resulting in overexpression of a native RPE enzyme, (iv) an exogenous polynucleotide sequence encoding an exogenous XPDH enzyme; (v) an exogenous polynucleotide sequence encoding an exogenous XKS enzyme; and (iv) an exogenous polynucleotide sequence encoding an exogenous XDH enzyme.

[0065] The final step in the xylitol pathway, from xylitol 5-phosphate to xylitol, requires a phosphatase enzyme. The *Saccharomyces cerevisiae* PYP1 (polyol phosphatase 1) gene encodes a sugar alcohol phosphatase that hydrolyzes sorbitol-6-phosphate, ribitol-5-phosphate, and (D)-glycerol-3-phosphase (Xu et al., "Discovery and functional characterization of a yeast sugar

alcohol phosphatase," ACS Chem. Biol., 13, 2018, 3011-3020). PYP1 is a member of the haloacid dehalogenase (HAD)-like hydrolase superfamily (Kuznetsova, et a., "Functional diversity of haloacid dehalogenase superfamily phosphatases from *Saccharomyces cerevisiae*," J. Biol. Chem., 2015, 290, 18678-18698) and belongs to the enzyme class of sorbitol-6-phosphatases (Enzyme Commission (EC) 3.1.3.50). As xylitol 5-phosphate is a similar molecule to the known substrates of PYP1 it is demonstrated herein that one or more PYP-like enzymes or PYP orthologs have xylitol-5-phosphate phosphatase activity and can be used to increase xylitol production in the recombinant cells described herein. *E. coli* HxpA (hexitol phosphatase A) is a HAD-like enzyme belonging to EC 3.1.3.50 with a similar substrate profile to PYP1 (Kuznetsova et al., "Genome wide analysis of substrate specificities of the *Escherichia coli* haloacid dehalogenase-like phosphate family," 2006, J. Biol. Chem., 281, 36149-36161). Accordingly, it is also demonstrated herein that one or more HAD-like hydrolase enzymes or HAD-like hydrolase orthologs have xylitol-5-phosphate phosphatase activity and can be used to increase xylitol production in the recombinant cells describe here.

[0066] The recombinant cells described herein are capable of producing xylitol, include an exogenous polynucleotide sequence encoding a trehalase enzyme, and may be characterized by overexpression of a native enzyme with xylitol-5-phosphate phosphatase (X5PP) activity and/or include an exogenous polynucleotide sequence encoding a native or exogenous enzyme with xylitol-5-phosphate phosphatase (X5PP) activity. In general, he recombinant cell(s) including overexpression of an X5PP enzyme or expressing an exogenous X5PP enzyme produce more xylitol than an equivalent cell lacking the exogenous X5PP enzyme or lacking overexpression of the X5PP enzyme. The enzyme may be any suitable enzyme with X5PP activity. As used herein, "X5PP enzyme" and "X5PP" are interchangeable and refer to an enzyme with X5PP activity. Herein, "xylitol-5-phosphate phosphatase activity" and "X5PP activity" are used interchangeably and refer to the ability to catalyze the conversion of xylitol-5-phosphate to xylitol and phosphate. Suitable X5PP enzymes may include a divalent metal cation, for example, Mg^{2+}, Mn^{2+}, or Co^{2+}. Suitable enzymes with X5PP activity may include, but are not limited to, those classified under EC 3.1.3.50, for example, sugar alcohol phosphatases and HAD-like hydrolases. Polynucleotides encoding X5PP enzymes may be derived from any suitable source. For example, a polynucleotide encoding an X5PP enzyme may be derived from *Moniliella pollinis*, *Saccharomyces cerevisiae*, *Lachancea dasiensis*, *Tetrapisispora blattae*, *Saccharomyces pastorianus*, *Kazachstania Africana*, *Podospora comata*, *Geotrichum candidum*, *Ogattaea haglerorum*, *Debaryomyces fabryi*,

Monilinia fructicola, *Nadsonia fulvescens* var. elongata DSM 6958, *Escherichia coli*, *Wickerhamomyces ciferrii*, *Bacillus amyloliquefaciens*, and the like. The X5PP enzyme may be a polypeptide with an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:24, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, and 103. The X5PP enzyme may be a polypeptide with an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:24, 89, 90, 91, 92, 93, 97, and 99. The X5PP enzyme may be a polypeptide with an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to the amino acid sequence of at least one of SEQ ID NOs:24, 90, 91, 93, and 99. See for example, US Provisional Application No. 63/499,992, filed May 4, 2023, and incorporated herein by reference in its entirety.

[0067] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Moniliella pollinis* gene encoding the amino acid sequence of SEQ ID NO:74. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:74.

[0068] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Saccharomyces cerevisiae* gene encoding the amino acid sequence of SEQ ID NO:75. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:75.

[0069] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Lachancea dasiensis* gene encoding the amino acid sequence of SEQ ID NO:76. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:76.

[0070] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous

polynucleotide sequence that is or may be derived from a *Tetrapisispora blattae* gene encoding the amino acid sequence of SEQ ID NO:77. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:77.

[0071] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Saccharomyces pastorianus* gene encoding the amino acid sequence of SEQ ID NO:78. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:78.

[0072] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Kazachstania africana* gene encoding the amino acid sequence of SEQ ID NO:79. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:79.

[0073] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Podospora comata* gene encoding the amino acid sequence of SEQ ID NO:80. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:80.

[0074] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Geotrichum candidum* gene encoding the amino acid sequence of SEQ ID NO:81. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:81.

[0075] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Ogattaea haglerorum* gene encoding the amino acid sequence of SEQ ID NO:82. The exogenous polynucleotide sequence may encode an

amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:82.

[0076] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Debaryomyces fabryi* gene encoding the amino acid sequence of SEQ ID NO:83. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:83.

[0077] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Monilinia fructicola* gene encoding the amino acid sequence of SEQ ID NO:84. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:84.

[0078] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Nadsonia fulvescens* var. elongata DSM 6958 gene encoding the amino acid sequence of SEQ ID NO:85. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:85.

[0079] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from an *Escherichia coli* gene encoding the amino acid sequence of SEQ ID NO:86. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:86.

[0080] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Wickerhamomyces ciferrii* gene encoding the amino acid sequence of SEQ ID NO:87. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:87.

[0081] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Moniliella pollinis* gene encoding the amino acid sequence of SEQ ID NO:88. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:88.

[0082] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Bacillus amyloliquefaciens* gene encoding the amino acid sequence of SEQ ID NO:89. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:89.

[0083] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Saccharomyces cerevisiae* DOG2 gene encoding the amino acid sequence of SEQ ID NO:90. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:90.

[0084] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may comprise an exogenous polynucleotide sequence that is or may be derived from a *Saccharomyces cerevisiae* DOG1 gene encoding the amino acid sequence of SEQ ID NO:91. The exogenous polynucleotide sequence may encode an amino acid sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99%, identical to SEQ ID NO:91.

[0085] The enzyme with X5PP activity may be native to the host cell. For example, when the host organism is *M. pollinis*, the X5PP enzyme may be an enzyme with a sequence at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to at least one of SEQ ID NOs:24, 101, 105, or 106. The recombinant cell may comprise an exogenous polynucleotide encoding an X5PP enzyme with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to at least one of SEQ ID NOs:24, 101, 105, or 106. The recombinant cell may include a genetic modification that increases expression of an X5PP enzyme at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:24, 101, 105, or 106. The genetic modification may include, but is not limited to, insertion of additional copies of a nucleic acid encoding the native X5PP enzyme into the cell (e.g., integration of additional copies of the X5PP encoding polynucleotide into it non-native locus in the cell), insertion of a constitutive promoter upstream of the coding region of the native X5PP enzyme encoding gene in the genome of the host cell, and/or modification of the existing promoter upstream of the coding region of the native X5PP enzyme encoding gene in the genome of the host cell. One of skill in the art will recognize that expression of a native X5PP enzyme encoding gene may be increased by a number of methods known in the art and will be able to select and apply such methods as appropriate.

[0086] As used herein, "overexpression" refers to an expression level of a polypeptide that is higher than the expression level of the same polypeptide in the absence of a genetic modification or exogenous polynucleotide encoding said polypeptide in an equivalent cell.

[0087] The recombinant cells described herein capable of producing xylitol and including an exogenous polynucleotide sequence encoding a trehalase enzyme may also be characterized by overexpression of a ribulose 5-phosphate epimerase (RPE enzyme). The recombinant cells described herein capable of producing xylitol and including an exogenous polynucleotide sequence encoding a trehalase enzyme may also include an exogenous polynucleotide sequence encoding an X5PP enzyme and/or overexpress a native X5PP enzyme, and/or may include an exogenous polynucleotide encoding a native or exogenous RPE enzyme or may have a genetic modification resulting in overexpression of a native RPE enzyme, as described herein. In general, the recombinant cell(s) including overexpression of the RPE enzyme produce more xylitol than an equivalent cell lacking the RPE enzyme or lacking overexpression of the RPE enzyme.

[0088] The recombinant cells described herein are capable of producing xylitol, include an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide encoding a native or exogenous RPE enzyme or may have a genetic modification resulting in overexpression of a native RPE enzyme. The RPE enzyme may be any suitable enzyme with ribulose 5-phosphate epimerase activity. As used herein, "ribulose 5-phosphate epimerase activity" and "RPE activity" are used interchangeably and refer to the ability to catalyze the conversion of ribulose-5-phosphate to xylulose-5-phosphate. The enzyme with RPE activity may be native to the host cell or the RPE enzyme may be an exogenous RPE enzyme. For example, when the host organism is *M. pollinis*, the RPE enzyme may be an enzyme with a sequence at

least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to at least one of SEQ ID NOs:14 and 107. The recombinant cell may comprise an exogenous polynucleotide encoding an RPE enzyme with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to at least one of SEQ ID NOs:14 and 107. The recombinant cell may include a genetic modification that increases expression of an RPE enzyme at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:14 and 107. The genetic modification may include, but is not limited to, insertion of additional copies of a nucleic acid encoding the native RPE into the cell, insertion of a constitutive promoter upstream of the coding region of the native RPE gene in the genome of the host cell, and/or modification of the existing promoter upstream of the coding region of the native RPE gene in the genome of the host cell. One of skill in the art will recognize that expression of a native RPE gene may be increased by a number of methods known in the art and will be able to select and apply such methods as appropriate. See for example, PCT Application No. PCT/US2023/066631 filed May 5, 2023.

[0089] The recombinant cells described herein are capable of producing xylitol, include an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide sequence encoding a xylitol-phosphate dehydrogenase (XPDH) enzyme. The exogenous polynucleotide sequence may be an exogenous xylitol-phosphate dehydrogenase (XPDH) gene. A recombinant cell described herein capable of producing xylitol has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide sequence encoding an X5PP enzyme and/or overexpress a native X5PP enzyme, may include an exogenous polynucleotide sequence encoding an XPDH enzyme, and/or may include an exogenous polynucleotide encoding a native or exogenous RPE enzyme or may have a genetic modification resulting in overexpression of a native RPE enzyme, as described herein.

[0090] A "xylitol-phosphate dehydrogenase gene" and an "XPDH gene" are used interchangeably herein and refer to any gene or polynucleotide that encodes a polypeptide with xylitol-phosphate dehydrogenase activity. As used herein, "xylitol-phosphate dehydrogenase activity" refer to the ability to catalyze the conversion of xylulose-5-phosphate and NADPH or NADH to xylitol 5-phosphate and $NADP^+$ or NAD^+. The XPDH gene may be derived from any suitable source. For example, the XPDH gene may be derived from *Clostridium difficile*, *Lactobacillus rhamnosus*, *Bacillus halodurans*, *Alkalihalobacillus ligniniphilus*, *Jeotgalibacillus*

soli, *Heyndrickxia sporothermodurans*, *Clostridium fungisolvens*, or *Neobacillus cucumis*. The XPDH gene may encode an amino acid at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to the amino acid sequence of at least one of SEQ ID NOs:5, 108, 109, 110, 111, 112, 113, 114, 115, and 116. The XPDH gene may encode an amino acid at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to the amino acid sequence of at least one of SEQ ID NOs:5, 110, 111, and 114. See for example, PCT Application No. PCT/US2023/066629, filed May 5, 2023, which is incorporated herein in its entirety.

[0091] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Clostridium difficile* gene encoding the amino acid of SEQ ID NO:108. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:108.

[0092] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Clostridium difficile* gene encoding the amino acid of SEQ ID NO:109. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:109.

[0093] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Lactobacillus rhamnosus* gene encoding the amino acid of SEQ ID NO:5. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:5.

[0094] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Bacillus halodurans* gene encoding the amino acid of SEQ ID NO:110. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:110.

[0095] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Alkalihalobacillus ligniniphilus* gene encoding the amino acid of SEQ ID NO:111. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:111.

[0096] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Jeotgalibacillus soli* gene encoding the amino acid of SEQ ID NO:112. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:112.

[0097] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Heyndrickxia sporothermodurans* gene encoding the amino acid of SEQ ID NO:113. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:113.

[0098] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Clostridium fungisolvens* gene encoding the amino acid of SEQ ID NO:114. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:114.

[0099] The recombinant cell is capable of producing xylitol, includes an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous polynucleotide that is, or may be derived from, a *Neobacillus cucumis* gene encoding the amino acid of SEQ ID NO:116. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:116.

[0100] The recombinant cells described herein are capable of producing xylitol, include an exogenous polynucleotide sequence encoding a trehalase enzyme, and may include an exogenous

polynucleotide sequence encoding a xylulokinase (XKS) enzyme. A recombinant cell described herein capable of producing xylitol and having an exogenous polynucleotide sequence encoding a trehalase enzyme may additionally include an exogenous polynucleotide sequence encoding an X5PP enzyme and/or overexpress a native X5PP enzyme, an exogenous polynucleotide sequence encoding an XKS enzyme, and/or include an exogenous polynucleotide encoding a native or exogenous RPE enzyme or may have a genetic modification resulting in overexpression of a native RPE enzyme, as described herein. The exogenous polynucleotide sequence may be an exogenous xylulose sugar phosphatase (XKS) gene.

[0101] A "xylulokinase gene" and an "XKS gene" are used interchangeably herein and refer to any gene or polynucleotide that encodes a polypeptide with xylulokinase activity. As used herein, "xylulokinase activity" refer to the ability to catalyze the conversion of xylulose-5-phosphate and ADP to xylulose and ATP. The XKS gene may be derived from any suitable source. For example, the XKS gene may be derived from *Saccharomyces cerevisiae*. The XKS gene may encode an amino acid at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to the amino acid sequence of at least one of SEQ ID NOs:104 and 103. Additional description of recombinant cells capable of producing xylitol and including a polypeptide with xylulokinase activity is provided in PCT Application No. PCT/US2023/066627, filed May 5, 2023, which is incorporated herein by reference in its entirety.

[0102] The recombinant cell is capable of producing xylitol, has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide that is, or may be derived from, a *Saccharomyces cerevisiae* DOG1 sugar phosphatase gene encoding the amino acid of SEQ ID NO:104. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:104.

[0103] The recombinant cell is capable of producing xylitol, has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide that is, or may be derived from, a *Saccharomyces cerevisiae* DOG2 sugar phosphatase gene encoding the amino acid of SEQ ID NO:103. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:103.

[0104] The recombinant cells described herein are capable of producing xylitol, have an exogenous polynucleotide sequence encoding a trehalase enzyme, and an exogenous

polynucleotide sequence encoding a xylitol dehydrogenase (XDH) enzyme. A recombinant cell described herein capable of producing xylitol and having an exogenous polynucleotide sequence encoding a trehalase enzyme may additionally include an exogenous polynucleotide sequence encoding a xylitol dehydrogenase (XDH) enzyme, an exogenous polynucleotide encoding an XKS enzyme, an exogenous polynucleotide sequence encoding an X5PP enzyme and/or overexpress a native X5PP enzyme, an exogenous polynucleotide sequence encoding an XKS enzyme, and/or include an exogenous polynucleotide encoding a native or exogenous RPE enzyme or may have a genetic modification resulting in overexpression of a native RPE enzyme, as described herein. The exogenous polynucleotide sequence may be an exogenous XDH gene.

[0105] A "xylitol dehydrogenase gene" and an "XDH gene" are used interchangeably herein and refer to any gene or polynucleotide that encodes a polypeptide with xylitol dehydrogenase activity. As used herein, "xylitol dehydrogenase activity" refer to the ability to catalyze the conversion of xylulose and NADH or NADPH to xylitol and NAD^+ or $NADP^+$. The XDH gene may be derived from any suitable source. For example, the XDH gene may be derived from *Pichia stipitis*, *Rhodobacteraceae bacterium*, or *Bemisia argentofolii*. The XDH gene may encode an amino acid at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to the amino acid sequence of at least one of SEQ ID NOs:117, 118, and 119. Additional description of recombinant cells capable of producing xylitol and including a polypeptide with xylulokinase activity and a polypeptide with xylitol dehydrogenase activity is provided in PCT Application No. PCT/US2023/066627, filed May 5, 2023, which is incorporated herein by reference in its entirety.

[0106] The recombinant cell is capable of producing xylitol, has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide that is, or may be derived from, a cofactor switched *Pichia stipitis* XDH gene encoding the amino acid of SEQ ID NO:117. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:117.

[0107] The recombinant cell is capable of producing xylitol, has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide that is, or may be derived from, a *Rhodobacteraceae bacterium* SDR family oxidoreductase gene encoding the amino acid of SEQ ID NO:118. The exogenous polynucleotide may encode an amino acid

sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:118.

[0108] The recombinant cell is capable of producing xylitol, has an exogenous polynucleotide sequence encoding a trehalase enzyme and may include an exogenous polynucleotide that is, or may be derived from, a *Bemisia argentofolii* (Silverleaf Whitefly) ketose reductase (sorbitol dehydrogenase) gene encoding the amino acid of SEQ ID NO:119. The exogenous polynucleotide may encode an amino acid sequence with at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:119.

[0109] The exogenous polynucleotides in the recombinant cells described herein may be under the control of a promoter. For example, the exogenous nucleic acid may be operably linked to a heterologous or artificial promoter. Suitable promoters are known and described in the art. Promoters may include, but are not limited to, pyruvate decarboxylase promoter (PDC), translation elongation factor 2 promoter (TEF2), SED1, alcohol dehydrogenase 1A promoter (ADH1), hexokinase 2 promoter (HXK2), FLO5 promoter, pyruvate kinase 1 promoter (PYK1p; SEQ ID NO:6); 6-phosphogluconate dehydrogenase promoter (6PGDp; SEQ ID NO:120); glyceraldehyde-3-phosphate dehydrogenase promoter (TDH3p; SEQ ID NO:121); translational elongation factor 1 promoter (TEFp; SEQ ID NO:122); phosphoglucomutase 1 promoter (PGM1p; SEQ ID NO:123); 3-phosphoglycerate kinase promoter (PGK1p; SEQ ID NO:22); enolase promoter (ENO1p ; SEQ ID NO:124); asparagine synthetase promoter (ASNSp; SEQ ID NO:125); 50S ribosomal protein L1 promoter (RPLAp; SEQ ID NO:126); RPL16B (SEQ ID NO:127), glycerol-3-phosphate dehydrogenase 2a promoter (GPDIIap; SEQ ID NO:138); glycerol dehydrogenase NADP(H) 1 promoter (GDN1p; SEQ ID NO:139); erythritol-P dehydrogenase 1 promoter (EPDH1; SEQ ID NO:140), translation elongation factor 1A like gene 7 promoter (TEF7p; SEQ ID NO:141); heat shock protein 90 promoter (HSP90p; SEQ ID NO:142); translation elongation factor 1A like gene 4 promoter (TEF4p; SEQ ID NO:143); translation elongation factor 2A like gene 6 promoter (TEF6p; SEQ ID NO:144); transaldolase 1 promoter (TAL1p; SEQ ID NO:145); citrate synthase 1 promoter (CIT1p; SEQ ID NO:146); endoplasmic reticulum chaperone BiP (KAR2p; SEQ ID NO:147); heat shock protein 88 promoter (HSP88p; SEQ ID NO:148); heat shock protein 70 promoter (HPS70p; SEQ ID NO:149); tubulin alpha-1 chain promoter (TUB1p; SEQ ID NO:150); glyceraldehyde-3-phosphate dehydrogenase 2 promoter (TDH2p; SEQ ID NO:151); translation elongation factor 1A like gene 5 promoter (TEF5p; SEQ ID NO:152);

translation elongation factor 3 promoter (TEF3p; SEQ ID NO:153); thioredoxin peroxidase promoter (TPXp; SEQ ID NO:154); alginate lyase promoter (ALPp; SEQ ID NO:155); thiazole biosynthetic 4 promoter (THI4p; SEQ ID NO:156); phosphoglucomutase/phospomannomutase promoter (PMMp; SEQ ID NO:157); fructose-bisphosphate aldolase 1 promoter (FBA1p; SEQ ID NO:158); alcohol dehydrogenase 1 promoter (ADH1p; SEQ ID NO:159); and extracellular endoglucanase 1 promoter (GLX1p; SEQ ID NO:160).

[0110] The exogenous nucleic acids in the recombinant cells described herein may be under the control of a terminator. For example, the exogenous nucleic acid may be operably linked to a heterologous or artificial terminator. Suitable terminators are known and described in the art. Terminators may include, but are not limited to, GAL10 terminator, PDC terminator, transaldolase terminator (TAL) 6PGD terminator (6PGDt; SEQ ID NO:70); ASNS terminator (ASNSt; SEQ ID NO:128); ENO1 terminator (ENO1t; SEQ ID NO:129); hexokinase 1 terminator (HXK1t; SEQ ID NO:130); PGK1 terminator (PGK1t; SEQ ID NO:131); PGM1 terminator (PGM1t; SEQ ID NO:132); PYK1 terminator (PYK1t; SEQ ID NO:15); RPLA terminator (RPLAt: SEQ ID NO:133); transaldolase 1 terminator (TAL1t; SEQ ID NO:134); TDH3 terminator (TDH3t; SEQ ID NO:135); translation elongation factor 2 terminator (TEF2t; SEQ ID NO:18); triosephosphate isomerase 1 terminator (TPI1t; SEQ ID NO:136); MpTEF1 (SEQ ID NO:137); TEF7 terminator (TEF7t; SEQ ID NO:161); HSP90 terminator (HSP90t; SEQ ID NO:162); TEF4 terminator (TEF4t; SEQ ID NO:163); TEF6 terminator (TEF6t; SEQ ID NO:164); CIT1 terminator (CIT11t; SEQ ID NO:165): KAR2 terminator (KAR2t; SEQ ID NO:166); HPS88 terminator (HSP88t; SEQ ID NO:167); HSP70 terminator (HSP70t; SEQ ID NO:168); TUB1 terminator (TUB1t; SEQ ID NO:169); TKL1 terminator (TAL1t; SEQ ID NO:170); TDH2 terminator (TDH2t; SEQ ID NO:171); TEF5 terminator (TEF5t; SEQ ID NO:172); TGL2 terminator (TGL2t; SEQ ID NO:173); TEF3 terminator (TEF3t; SEQ ID NO:174); TPX terminator (TPXt; SEQ ID NO:175); ALP terminator (ALPt; SEQ ID NO:176); plasma membrane ATPase terminator (PMA1t; SEQ ID NO:177); THI4 terminator (THI4t; SEQ ID NO:178); glucose-6-phosphate isomerase 1 terminator (PGI1t; SEQ ID NO:179); PMM terminator (PMMt; SEQ ID NO:180); FBA1 terminator (FBA1t; SEQ ID NO:181); ADH1 terminator (ADH1t; SEQ ID NO:182); sodium/potassium transporting ATPase alpha chain terminator (ATP1t; SEQ ID NO:183); and GLX1 terminator (GLX1t; SEQ ID NO:184).

[0111] A promoter or terminator is "operably linked" to a given polynucleotide (e.g., a gene) if its position in the genome or expression cassette relative to said polynucleotide is such that the promoter or terminator, as the case may be, performs its transcriptional control function.

[0112] The polypeptides described herein may be provided as part of a construct. As used herein, the term "construct" refers to recombinant polynucleotides including, without limitation, DNA and RNA, which may be single-stranded or double-stranded and may represent the sense or the antisense strand. Recombinant polynucleotides are polynucleotides formed by laboratory methods that include polynucleotide sequences derived from at least two different natural sources or they may be synthetic. Constructs thus may include new modifications to endogenous genes introduced by, for example, genome editing technologies. Constructs may also include recombinant polynucleotides created using, for example, recombinant DNA methodologies. The construct may be a vector including a promoter operably linked to the polynucleotide encoding a polypeptide as described herein. As used herein, the term "vector" refers to a polynucleotide capable of transporting another polynucleotide to which it has been linked. The vector may be a plasmid, which refers to a circular double-stranded DNA loop into which additional DNA segments may be integrated.

[0113] The disclosure also provides methods for reducing trehalose in fermentation for the production of xylitol. In general, the method includes contacting a substrate with an engineered cell capable of producing xylitol. During fermentation, a trehalase enzyme is added to the fermentation broth to reduce and/or eliminate trehalose byproduct from the fermentation broth. Without being bound to any particular theory or mode of action, trehalase enzyme catalyzed breakdown of trehalose into two molecules of glucose will increase the glucose concentration available to the engineered cell for the production of xylitol and the fermentation process may also have an increased titer and/or yield relative to xylitol titer and/or yield in an equivalent process without the trehalase enzyme.

[0114] The trehalase enzyme may be added to the fermentation broth exogenously, *e.g.*, not generated *in situ* in the fermentation reaction, as the fermentation process proceeds. The trehalase enzyme may be added after the engineered cells have passed growth phase. The trehalase enzyme may be added one, two, three, or more times during the fermentation. The trehalase enzyme may be continuously added to the fermentation broth.

[0115] The trehalase enzyme may be secreted from the engineered cell. For example, the fermentation method may include the step of fermenting a substrate using the genetically

engineered yeasts described herein to produce xylitol, whereby trehalase enzyme expressed by the engineered yeast is excreted into the fermentation broth and the concentration of trehalose is reduce.

[0116] The fermentation method can include additional steps, as would be understood by a person skilled in the art. Non-limiting examples of additional process steps include maintaining the temperature of the fermentation broth within a predetermined range, adjusting the pH during fermentation, and isolating the xylitol from the fermentation broth. The fermentation process may be a fully aerobic or a partially aerobic process.

[0117] The fermentation method can be run using a suitable fermentation substrate. The substrate of the fermentation method can include glucose, sucrose, galactose, mannose, molasses, xylose, fructose, hydrolysates of starch, lignocellulosic hydrolysates, or a combination thereof. One skilled in the art will recognize what fermentation substrate is suitable for a given fermentation organism and system.

[0118] The fermentation process can be run under various conditions. The fermentation temperature, i.e., the temperature of the fermentation broth during processing, may be ambient temperature. Alternatively, or additionally, the fermentation temperature may be maintained within a predetermined range. For example, the fermentation temperature can be maintained in the range of 25 °C to 45 °C, 30 °C to 40 °C, or 32 °C to 37 °C, preferably about 35 °C. However, a skilled artisan will recognize that the fermentation temperature is not limited to any specific range or temperature recited herein and may be modified as appropriate.

[0119] The fermentation process can be run within certain oxygen uptake rate (OUR) ranges. The volumetric OUR of the fermentation process can be in the range of 5-80, 10-75, 15-70, 20-60, 30-50, or 40-50 mmol O_2/(L • h). In some embodiments, the specific OUR can be in the range of 0.05 to 10, 0.1 to 9, 0.5 to 8, 1.0 to 7, 1.5 to 6, 2 to 5, or 2.5 to 4 mmol O_2/(g cell dry weight • h). However, the volumetric or specific OURs of the fermentation process are not limited to any specific rates or ranges recited herein.

[0120] The fermentation process can be run at various cell concentrations. In some embodiments, the cell dry weight at the end of fermentation can be 5 to 40, 8 to 30, or 10 to 20 g cell dry weight/L. Further, the pitch density or pitching rate of the fermentation process can vary. In some embodiments, the pitch density can be 0.05 to 11, 0.1 to 10, or 0.25 to 8 g cell dry weight/L.

[0121] The initial dextrose concentration of the fermentation may be at least 100, 200, 250, 300, 350, or at least 400 g/L dextrose when run as a batch fermentation. The initial dextrose concentration may be between 100 to 500, 150 to 450, 400 to 200, or 250 to 350 g/L when run as a batch fermentation. When run in a fed batch fermentation process, the dextrose concentration may be at least 100, 200, 250, 300, 350, 400, or at least 450 g/L.

[0122] The fermentation process can be run as a dextrose-fed batch. Further, the fermentation process can be a batch process, continuous process, or semi-continuous process, as would be understood by a person skilled in the art.

[0123] The fermentation process can be associated with various characteristics, such as, but not limited to, fermentation production rate, pathway fermentation yield, final titer, and peak fermentation rate. These characteristics can be affected by the selection of the yeast and/or genetic modification of the yeast used in the fermentation process. These characteristics can be affected by adjusting the fermentation process conditions. These characteristics can be adjusted via a combination of yeast selection or modification and the selection of fermentation process conditions.

[0124] The xylitol production rate of the process may be at least 0.2, 0.3, 0.5, 0.75, or at least 1.0 g L^{-1} h^{-1}. The final xylitol titer of the process may be at least 5, 10, 20, 30, 50, 75, or 100 g/L.

[0125] The xylitol yield of the process may be at least 25, at least 30, at least 35, at least 37, at least 38, or at least 40 percent. The xylitol yield of the process may be higher than the xylitol yield of an equivalent process run in the absence of the trehalase enzyme. For example, the xylitol yield of the process may be higher than the xylitol yield of an equivalent process using an equivalent engineered cell lacking the polynucleotide sequence encoding the trehalase enzyme.

[0126] The trehalose titer of the process may be reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% relative to an equivalent fermentation process run without a trehalase enzyme. The trehalase titer of the process may be below instrument detection limits. The trehalase titer may be 0 g/L.

EXAMPLES

[0127] The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being

limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

[0128] Throughout the Examples, strain numbering and sequence identification numbers are used consistently. For example, strain 1-8 in Example 2 is the same as strain 1-8 in Example 3, etc.

Example 1: Trehalase Sequences

[0129] In a fermentation reaction, trehalose can accumulate in the fermentation broth. The proposed solution for removing trehalose from the fermentation broth during the production of xylitol is to use a trehalase enzyme in the fermentation both. In these examples trehalase enzymes outlined in Table 2 will be utilized.

Table 2.

Trehalase	SEQ ID NO:
M. grisea	25
C. glabrata	26
Moniliella pollinis XTH "full"	27
Moniliella pollinis XTH "short"	28

[0130] The trehalase may include or may be engineered to include a secretion signal sequence such that the trehalase is secreted from the cell and into the fermentation broth. The trehalase may have its own native secretion signal or it may be engineered to include an exogenous signal sequence. Table 3 lists various secretion signals used in these examples.

Table 3.

Name	Gene	Source	Description	SEQ ID NO:
MpProt2	RCSR16341	*M. pollinis*	Cerevisin, yeast proteinase B (EC 3.4.21.48)	29
MpGla	RCSR09567	*M. pollinis*	Glucoamylase (EC 3.2.1.3)	30
MpLac	RCSR10913	*M. pollinis*	Laccase (EC 1.10.3.2)	31
MFalpha2	YGL089C	*S. cerevisae*	Mating pheromone alpha-factor	32

Example 2: Genetically Modified Moniliella pollinis Strains

Strain 1-1

[0131] Strain 1-1 is the *Moniliella pollinis* host strain "*Moniliella tomentosa* var *pollinis* TCV364" described in US 6,440,712, which is incorporated herein by reference in its entirety, and deposited under the Budapest Treaty at BCCM/MUCL (Belgian Coordinated Collections of Micro-organisms/Mycothèque de l'Université Catholique de Louvain by Eridania Béghin Say, Vilvoorde R&D Centre, Havenstraat 84, B-1800 Vilvoorde) on March 28, 1997 under number MUCL40385.

Strain 1-2

[0132] Strain 1-1 was transformed with SEQ ID NO:2 and SEQ ID NO:1 by first protoplasting the parent strain by adding an enzyme mixture containing 0.6M $MgSO_4$, 7.5 g/L driselase, and 12.5 g/L *Trichoderma harzianum* lysing enzyme to a mycelial pellet of the parent strain. Protoplasts were then pelleted, washed with 0.6M $MgSO_4$, and resuspended in STC medium (0.6M sucrose, 50 mM CaCl2, 10 mM Tris-HCl, pH 7.5). Bipartite transformations were prepared by adding 100 µg single stranded salmon sperm DNA and 1.5 to 5 µg each of the 5' and 3' DNA transformation fragments (3-10 µg total) to approximately 200 µL protoplast mixture (10^8 cells/mL). 1 mL 50% PEG in STC medium was then added to the salmon sperm DNA, transformation DNA, and protoplast mixture and the resulting combination was incubated for 15 minutes at room temperature. Following incubation, recovery broth (0.4M sucrose, 1 g/L yeast extract, 1 g/L malt extract, 10 g/L glucose, pH 4.5) was added to the mixture and incubated at 27 ºC, 100 rpm, for 16 to 24 hours. Following the incubation, protoplasts were pelleted by centrifugation and resuspended in 1 mL PBS. The resuspended protoplasts were plated on PDA + 250 mg/L geneticin (G418) selection plates and incubated at 35 ºC for at least 2 days until transformants grew. Resulting transformants were streaked for single colony isolation on PDA + geneticin (G418) plates and single colonies were selected. Selected colonies were evaluated by colony PCR for integration of two copies of the *L. rhamnosus* XPDH sequence. A PCR verified isolate was designated strains 1-2.

[0133] SEQ ID NO:1 contains (i) 5' flanking DNA for targeted chromosomal integration into the ER1 locus (SEQ ID NO:3); (ii) a polynucleotide sequence, SEQ ID NO:4, encoding the XPDH homolog from *Lactobacillus rhamnosus* of SEQ ID NO:5, under the control of the PYK1 promoter of SEQ ID NO:6 and the PGD terminator of SEQ ID NO:7; and (iii) a 5' portion of the G418

selectable marker (SEQ ID NO:8).SEQ ID NO:2 contains (i) a 3' portion of the G418 selectable marker (SEQ ID NO:9); (ii) a polynucleotide sequence, SEQ ID NO:4, encoding the XPDH homolog from *Lactobacillus rhamnosus* of SEQ ID NO:5 , under the control of the PYK1 promoter of SEQ ID NO:6 and the PGD terminator of SEQ ID NO:7; and (iii) a 3' flanking DNA for targeted chromosomal integration into the ER1 locus (SEQ ID NO:10).

Strain 1-3

[0134] Strain 1-2 was transformed with SEQ ID NO:11 and SEQ ID NO:12 using the protocol outlined above. The transformation fragment of SEQ ID NO:11 contained, in order, a 5' ER3 flanking sequence (SEQ ID NO:13), a MpPYK1 promoter (SEQ ID NO:6), a gene encoding the *M. pollinis* RPE2 polypeptide of SEQ ID NO:14, a MpPYK terminator (SEQ ID NO:15), and a 5' portion of a zeocin resistance gene expression cassette (SEQ ID NO:16). The transformation fragment of SEQ ID NO:12 contained, in order, a 3' portion of a zeocin resistance gene expression cassette (SEQ ID NO:17), an MpTEF2 terminator (SEQ ID NO:18), and a 3' ER3 flanking sequence (SEQ ID NO:19). Resulting transformants were streaked for single colony isolation on PDA + zeocin plates and single colonies were selected. Selected colonies were evaluated by colony PCR for integration of the *M. pollinis* RPE2 sequence. A PCR verified isolate was designated strains 1-3.

Strain 1-4

[0135] UV mutagenesis (using a Hoefer UV Crosslinker at an energy of 360 uJ/cm^3) and selection of strain 1-3 was used to generate a *Moniliella pollinis* strain with reduced foaming during shake flask fermentation. Strains with low-foaming phenotypes were selected based on visual evaluation of foaming in a shake flask fermentation compared to foaming on the parent 1-3 strain. The resulting low-foaming strain, containing two copies of an exogenous polynucleotide sequence encoding the XPDH of SEQ ID NO:5 integrated at the ER1 locus and one copy of a polynucleotide sequence encoding the RPE of SEQ ID NO:14 integrated at the ER3 locus, was designated 1-4.

Strain 1-5

[0136] Strain 1-4 was transformed with the Cre recombinase plasmid of SEQ ID NO:20 using the transformation method outlined above. The resulting transformants were evaluated by colony

PCR for removal of the G418 and zeocin resistance selection marker. A PCR verified isolate was designated strain 1-5.

Strain 1-6

[0137] Strain 1-5 was grown non-selectively on YPD plates to allow for the loss of the plasmid of SEQ ID NO:20. Biomass was struck for single colonies and evaluated by PCR to confirm loss of the plasmid. A PCR verified isolate was designated strain 1-6.

Strain 1-7

[0138] Strain 1-6 was transformed with SEQ ID NO:21 and SEQ ID NO:11 using the transformation method outlined above. SEQ ID NO:21 contained, in order, a 3' portion of a zeocin resistance gene expression cassette (SEQ ID NO:17), a MpPGK1 promoter (SEQ ID NO:22), a gene (SEQ ID NO:23) encoding the *M. pollinis* X5PP polypeptide of SEQ ID NO:24, a Mp6PGD terminator (SEQ ID NO:7), and a 3' ER3 flanking sequence (SEQ ID NO:19). Resulting transformants were streaked for single colony isolation on PDA + zeocin plates and single colonies were selected. Selected colonies were evaluated by colony PCR for integration of the indicated sequence. A PCR verified isolate was designated strain 1-7.

Strain 1-8

[0139] To remove the zeocin resistance selection marker, strain 1-7 was transformed with the Cre recombinase plasmid of SEQ ID NO:20 using the transformation method outlined above. The resulting transformants were evaluated by colony PCR for removal of the zeocin resistance selection marker. A PCR verified isolate was designated strain 1-8.

Strains 1-9 through 1-26

[0140] Strain 1-8 was transformed according to Table 4 with SEQ ID NO:67 and one of SEQ ID NOs:49-66 using the transformation method outlined above. SEQ ID NO:67 contained, in order, a 5' gpdIIB flanking region (SEQ ID NO:86), and a 5' portion of a G418 resistance gene expression cassette (SEQ ID NO:8). Each of SEQ ID NOs:49-66 contained, in order, a 3' portion of a G418 resistance gene expression cassette (SEQ ID NO:9), am MpTDH3 promoter (SEQ ID NO:121), a nucleotide sequence encoding one of SEQ ID NOs: 27, 28, or 33-48, an Mp6PGD terminator (SEQ ID NO:7), and a 3' gpdIIB flanking region (SEQ ID NO:87). Resulting

transformants were streaked for single colony isolation on PDA + 250 mg/L geneticin (G418) plates and single colonies were selected. Selected colonies were evaluated by colony PCR for integration of the indicated sequence. PCR verified isolates were designated as outlined in Table 4.

[0141] For example, strain 1-8 was transformed with SEQ ID NO:67 and SEQ ID NO:49 using the transformation method outline above. SEQ ID NO:49 contained, in order, a 3' portion of a G418 resistance gene expression cassette (SEQ ID NO:9), am MpTDH3 promoter (SEQ ID NO:121), a nucleotide sequence encoding SEQ ID NO:33, an Mp6PGD terminator (SEQ ID NO:7), and a 3' gpdIIB flanking region (SEQ ID NO:87). Resulting transformants were streaked for single colony isolation on PDA + 250 mg/L geneticin (G418) plates and single colonies were selected. Selected colonies were evaluated by colony PCR for integration of the sequence encoding the polypeptide of SEQ ID NO:33. PCR verified isolates were designated strains 1-9a, 1-9b, 1-9c, and 1-9d.

Table 4.

Strain	Parent Strain	First Bipartite Fragment				Second Bipartite Fragment
		Secretion Signal SEQ ID NO:	Trehalase SEQ ID NO:	Signal + Trehalase Trehalase SEQ ID NO:	Plasmid SEQ ID NO:	Plasmid SEQ ID NO:
1-9a-d	1-8	29	25	33	49	67
1-10a-d	1-8	30	25	34	50	67
1-11a-d	1-8	31	25	35	51	67
1-12a-d	1-8	32	25	36	52	67
1-13a-d	1-8	29	26	37	53	67
1-14a-d	1-8	30	26	38	54	67
1-15a-d	1-8	31	26	39	55	67
1-16a-d	1-8	32	26	40	56	67
1-17a-d	1-8	-	28	28	57	67
1-18a-d	1-8	-	27	27	58	67
1-19a-d	1-8	29	27	41	59	67
1-20a-d	1-8	30	27	42	60	67

1-21a-d	1-8	31	27	43	61	67
1-22a-d	1-8	32	27	44	62	67
1-23a-d	1-8	29	28	45	63	67
1-24a-d	1-8	30	28	46	64	67
1-25a-d	1-8	31	28	47	65	67
1-26a-d	1-8	32	28	48	66	67

Example 3: Deep Well Plate Assay

[0142] Strains from Example 2 were added to 48 well flower plates containing 1 mL rich medium (170 g/L glucose, 10 g/L yeast extract) and incubated for 44 hours at 900 RPM and 30 ºC. 20 µl of the resulting culture was transferred to 48 well flower plates containing production medium (Table 5) in triplicate (plates A, B, and C). Control strain 1-8 was also added to each plate. Plate A was removed at 48 hours, plate B at 72 hours, and plate C at 90 hours. Plate C (90 hours) was analyzed for xylitol, DP2 sugar, and trehalose by high performance liquid chromatography with refractive index detector. Results are reported in Table 6 and FIG. 2.

Table 5: Production Medium

Component	Concentration (units)
Glucose	300 (g/L)
KH2PO4	1.27 (g/L)
(NH4)2HPO4	0.13 (g/L)
(NH4)2SO4	1.80 (g/L)
Urea	2.85 (g/L)
Citric Acid	200.0 (mg/L)
MgSO4-7H2O	690.2 (mg/L)
FeSO4-7H2O	32.35 (mg/L)
ZnSO4-7H2O	4.40 (mg/L)
MnSO4-H2O	0.53 (mg/L)
CuSO4-5H2O	0.45 (mg/L)
CaCl2-2H2O	91.60 (mg/L)

Na2SO4	154.50 (mg/L)
Thiamine-HCl	10 (mg/L)
Choline-Cl	100 (mg/L)
Antifoam CF-32	1.00 (g/L)

Table 6.

Strain	Trehalase (SEQ ID NO:)	Xylitol		DP2		Trehalose	
		(g/L)	Standard Dev.	(g/L)	Standard Dev.	(g/L)	Standard Dev.
1-9a-d	MpProt2_MgTreA (33)	107.2	9.4	3.2	0.5	2.4	0.1
1-10a-d	MpGla_MgTreA (34)	108.4	11.3	2.9	1	1.9	0.8
1-11a-d	MpLac_MgTreA (35)	112.1	3.4	1.3	0.4	0.0	0.0
1-12a-d	Mfalpha2_MgTreA (36)	109.7	8.8	1.6	0.9	0.9	0.8
1-13a-d	MpProt2_CgATH1 (37)	107.7	6.5	3.6	0.7	2.9	0.7
1-14a-d	MpGla_CgATH1 (38)	106.5	3.5	2.4	1.4	1.6	1.3
1-15a-d	MpLac_CgATH1 (39)	105.1	7.3	2.6	1.6	1.6	1.3
1-16a-d	Mfalpha2_CgATH1 (40)	104	5	2.2	1.2	1.4	0.9
1-17a-d	MpXTH1short (28)	109.8	2.5	2.8	0.4	2.1	0.4
1-18a-d	MpXTH1 full (27)	92.2	35.7	3.1	0.9	2.3	0.7
1-19a-d	MpProt2_XTH1 full (41)	116.5	4.2	3.5	0.7	2.6	0.7
1-8	No trehalase control for strains 1-9 through 1-19	116.4	0.3	3	0	2.0	0.1
1-20a-d	MpGla_XTH1 full (42)	103.3	6.8	3.2	0.8	2.5	0.7
1-21a-d	MpLac_XTH1 full (43)	109.7	4.9	2.5	0.5	1.7	0.4
1-22a-d	Mfalpha2_XTH1 full (44)	101.1	15	3.3	1	2.6	1.0
1-23a-d	MpProt2_XTH1 short (45)	55.2	62.5	2.2	1.3	1.5	1.3
1-24a-d	MpGla_XTH1 short (46)	110	4.7	2.7	0.6	2.0	0.5
1-25a-d	MpLacc_XTH1 short (47)	102.9	5.9	3.2	1.1	2.3	1.0
1-26a-d	Mfalpha2_XTH1 short (48)	98.3	14	3.1	1.2	2.4	1.2
1-8	No trehalase control for strains 1-20 through 1-26	109.9	8.7	3	0.6	2.0	0.5

[0143] The results show that several of the strains had reduced DP2 sugars and trehalose relative to the control. For example, strains 1-11, 1-12, 1-14, 1-15, 1-16, 1-21, and 1-23 showed a reduction in both DP2 sugar and trehalose at 90 hours compared to control strain 1-8. These results demonstrate that secretion of a trehalase enzyme into the fermentation broth, for example by the use of a secretion signal, reduces the amount of trehalose in the fermentation broth.

Example 4: Shake Flask Fermentation Assay

[0144] Strains 1-9c, 1-11a-d, 1-12b-d, 1-14b, 1-14d, 1-15b, 1-15c, 1-16a, 1-16c, 1-16d, 1-17a, 1-17c, 1-18a, 1-18c, 1-20d, 1-21b, 1-22a, 1-23c, 1-24d, 1-25s, 1-25d, 1-26c, and 1-8 were run in shake flasks to assess glucose consumption as well as erythritol, xylitol, glycerol, trehalose, and ethanol production.

[0145] Strains were streaked out for biomass growth on YPD plates (bacteriological peptone 20g/L, yeast extract 10 g/L, glucose 20 g/L, and agar 15 g/L) and incubated at 30 °C for 48-72 hours. Cells from the incubated YPD plates were scraped into 40 mL rich medium (170 g/L glucose, 10 g/L yeast extract) in a 250 mL non-baffled flask. Cells were incubated at 30 °C and 250 rpm until the optical density (OD600) reached 15-20 to form the seed culture. Optical density is measured at a wavelength of 600 nm with a 1 cm path length cuvette using a model Genesys20 spectrophotometer (Thermo Scientific). The seed culture reached an OD600 between 15-20 in about 32-50 hours.

[0146] A 250 ml non-baffled flask containing 20mL production medium (Table 5) was inoculated with 0.4 mL of the seed culture to form the production culture. The production culture was incubated at 35 °C and 250 rpm. Samples were taken from the production culture after 48, 72, and 96 hours of incubation. Samples were analyzed for glucose, xylitol, erythritol, glycerol, trehalose, and ethanol by high performance liquid chromatography with refractive index detector. Results are shown in Table 7 and xylitol yield is reported in Table 8. Results are also shown in FIGS. 3 and 4. Trehalose titer was only measured for the 72- and 96-hour time points. Xylitol yield was calculated by dividing the concentration of xylitol (w/v) by the total glucose consumed at a given time point (w/v). For example, if the starting glucose was 100 g/L and at 48 hours 20 g/L of xylitol was produced and 20 g/L of glucose remained, the yield at the 48 hour time point would be the xylitol concentration at 48 hours (20 g/L) divided by the total consumed glucose at 48 hours (100 g/L – 20g/L), resulting in a yield of 25%.

[0147] Results show that several of the trehalase enzymes tested reduce trehalase titer and/or increased xylitol yield relative to the control strain 8-1. For example at 96 hours, strains 1-9c, 1-11a, 1-11b, 1-11c, 1-11d, 1-12b, 1-12c, 1-12d, 1-14b, 1-14d, 1-15b, 1-15c, 1-16a, 1-16c, 1-16d, 1-17a, 1-17c, 1-18c, 1-21b, 1-22a, 1-23c, 1-24d, and 1-26c all showed trehalase titers below the 2.45 (g/L) of control strain 1-8. Further, strains 1-11b, 1-16c, and 1-16d showed reduction of trehalase titer to below the detection limit of the HPLC. After 96 hours of fermentation, strains 1-9c, 1-11a, 1-11d, 1-12b, 1-12c, 1-12d, 1-14d, 1-15b, 1-15c, 1-16a, 1-16c, 1-16d, 1-17a, 1-17c, 1-20d, 1-21b, and 1-22a produced a higher yield of xylitol than the control strain 8-1. Strains 1-9c, 1-11a, 1-15b, 1-17c, and 1-20d showed a greater than 0.5% increase in yield, while strains 1-11d, 1-12d, 1-15c, 1-16c, and 1-22a showed greater than 1.0% increase in yield.

[0148] There is some sister-to-sister strain variability in several of the strains assayed. While a strain is PCR verified to contain the desired polynucleotide sequence, said sequence may not be integrated at the correct loci, it may have had multiple copies of the sequence integrated into its genome, or a frameshift or other mutation caused an individual sister to vary from the others. The results here suggest that similar transformation occurrences are present in these sisters. However, the results still demonstrate that expression of the encoded trehalase reduces trehalose concentration in the fermentation broth and increase xylitol yield.

Table 7.

Strain	Time Point (h)	Fermentation Broth Analyte (g/L)					Time Point (h)	Fermentation Broth Analyte (g/L)						Time Point (h)	Fermentation Broth Analyte (g/L)					
		Glucose	Xylitol	Erythritol	Glycerol	Ethanol		Glucose	Xylitol	Erythritol	Glycerol	Ethanol	Trehalose		Glucose	Xylitol	Erythritol	Glycerol	Ethanol	Trehalose
1-9c	48	150.71	28.14	8.84	20.14	19.20	72	15.68	86.00	22.69	19.56	28.15	4.59	96	0.08	108.23	24.76	9.37	15.49	1.12
1-11a	48	157.22	27.09	8.07	20.10	18.42	72	14.04	88.41	22.11	20.29	29.70	3.65	96	0.05	107.97	23.55	10.29	16.94	0.82
1-11b	48	185.52	21.31	5.65	13.88	17.44	72	37.05	79.79	15.58	13.65	34.97	3.37	96	0.18	104.18	17.08	11.19	25.04	n.a.
1-11c	48	170.98	24.90	8.03	13.64	17.55	72	21.69	83.86	22.55	12.88	32.25	4.02	96	0.12	102.57	24.89	8.23	19.57	0.25
1-11d	48	146.86	31.51	8.94	19.37	20.27	72	4.18	96.76	21.87	19.34	27.62	2.74	96	0.09	114.88	22.73	8.14	10.68	0.43
1-12b	48	131.09	36.21	9.44	14.17	26.37	72	4.20	96.76	18.38	12.39	32.17	2.98	96	0.14	106.21	19.40	8.37	16.95	0.40
1-12c	48	142.73	31.29	8.69	16.74	23.00	72	7.64	92.27	19.23	15.69	32.94	4.05	96	0.11	106.09	20.27	9.36	18.00	0.39
1-12d	48	136.54	35.96	9.24	14.29	25.19	72	4.35	98.81	18.99	11.75	32.58	3.35	96	0.09	109.15	20.11	7.52	16.72	0.33
1-14b	48	132.30	34.14	8.58	15.62	26.56	72	3.37	93.45	17.69	13.58	35.13	3.50	96	0.12	102.22	18.56	9.27	20.21	0.10
1-14d	48	173.05	24.09	6.44	16.58	18.64	72	30.34	82.65	16.90	16.39	30.89	4.85	96	0.13	107.00	18.93	12.60	19.10	0.40
1-15b	48	132.07	36.14	10.73	20.01	22.36	72	0.15	98.25	24.45	16.73	28.60	4.04	96	0.08	108.84	24.86	7.45	15.24	0.86
1-15c	48	166.88	23.12	6.73	22.53	16.38	72	29.68	79.67	19.04	24.04	24.93	5.47	96	0.07	110.59	21.85	10.97	12.34	1.29
1-16a	48	146.57	31.07	8.76	17.12	22.88	72	12.22	92.01	18.89	15.21	32.89	4.93	96	0.21	106.63	19.77	9.86	21.66	0.50
1-16c	48	152.43	28.48	8.28	19.29	19.62	72	26.16	84.64	19.78	19.49	27.09	4.02	96	0.11	109.71	22.41	10.54	17.50	n.a.
1-16d	48	138.63	32.75	9.27	17.25	23.27	72	8.00	92.79	19.41	16.07	32.47	4.58	96	0.13	106.93	20.40	10.10	20.44	n.a.
1-17a	48	143.73	32.14	8.89	17.25	23.19	72	10.47	92.44	19.11	15.36	32.27	5.64	96	0.09	107.10	20.01	9.62	19.22	2.16
1-17c	48	152.73	28.53	8.44	19.63	19.66	72	12.33	89.80	22.39	19.82	29.69	4.92	96	0.08	108.58	23.67	9.42	15.00	2.36

1-18a	48	169.77	24.03	9.29	14.10	17.82	72	42.31	72.76	24.34	11.90	28.28	7.06	96	3.01	96.77	29.96	6.69	19.23	5.37
1-18c	48	151.59	29.98	8.03	14.39	23.25	72	18.51	88.83	17.47	12.95	34.30	4.49	96	0.17	103.91	18.59	10.04	23.34	2.02
1-20d	48	144.16	30.47	8.49	18.33	22.58	72	10.37	91.01	19.43	18.78	29.45	5.21	96	0.06	107.77	20.08	9.60	17.40	2.76
1-21b	48	144.87	30.68	8.46	17.52	22.68	72	15.64	89.28	18.64	15.84	32.56	5.14	96	0.10	106.81	19.97	9.98	20.37	1.62
1-22a	48	155.63	27.61	7.75	18.32	19.66	72	34.93	81.58	18.29	16.73	28.39	5.28	96	0.14	110.04	20.51	10.59	20.55	2.12
1-23c	48	149.65	31.07	8.25	14.92	23.29	72	19.20	89.18	17.59	12.81	34.39	5.70	96	0.20	105.02	18.39	10.06	23.59	2.41
1-24d	48	135.38	37.48	10.48	17.06	22.19	72	0.15	101.08	23.04	13.84	31.12	3.42	96	0.07	105.00	23.90	10.08	18.72	1.99
1-25a	48	149.12	28.99	9.40	19.87	19.62	72	18.62	82.57	23.50	18.53	27.77	5.31	96	0.06	103.39	25.68	9.38	18.33	2.52
1-25d	48	130.37	33.78	9.92	22.76	21.07	72	15.52	87.21	21.11	21.48	26.27	4.75	96	1.27	100.61	22.58	18.96	17.32	4.38
1-26c	48	142.51	31.86	9.23	18.83	21.00	72	6.50	92.09	21.73	18.48	31.63	4.65	96	0.12	104.73	22.44	11.32	19.44	2.21
1-8	48	152.83	28.56	8.52	19.97	19.55	72	14.49	88.23	21.83	19.34	29.64	5.05	96	0.06	106.11	23.00	10.79	19.33	2.45

n.a. indicates the analyte was undetectable by the method used.

PT-1892-WO-PCT

Table 8: Xylitol Yield at 96 Hours

Strain	24-hour yield (%)	48-hour yield (%)	72-hour yield (%)	96-hour yield (%)
1-9c	6.6%	20.1%	31.2%	37.2%
1-11a	9.7%	20.2%	31.9%	37.1%
1-11b	12.9%	20.2%	31.4%	35.8%
1-11c	10.7%	20.7%	31.1%	35.3%
1-11d	11.1%	21.9%	33.7%	39.5%
1-12b	9.9%	22.6%	33.7%	36.5%
1-12c	10.0%	21.1%	32.6%	36.5%
1-12d	12.4%	23.3%	34.5%	37.5%
1-14b	8.7%	21.5%	32.5%	35.1%
1-14d	13.6%	20.4%	31.7%	36.8%
1-15b	9.8%	22.7%	33.8%	37.4%
1-15c	11.2%	18.6%	30.5%	38.0%
1-16a	10.3%	21.5%	33.0%	36.7%
1-16c	8.0%	20.5%	32.0%	37.7%
1-16d	9.3%	21.5%	32.8%	36.8%
1-17a	9.1%	21.8%	32.9%	36.8%
1-17c	8.4%	20.6%	32.2%	37.3%
1-18a	10.0%	19.8%	29.2%	33.6%
1-18c	9.2%	21.5%	32.6%	35.7%
1-20d	8.1%	20.7%	32.4%	37.0%
1-21b	8.1%	21.0%	32.4%	36.7%
1-22a	9.7%	20.4%	31.9%	37.8%
1-23c	9.4%	22.0%	32.8%	36.1%
1-24d	10.8%	24.1%	34.7%	36.1%
1-25a	8.2%	20.4%	30.3%	35.5%
1-25d	8.5%	21.0%	31.6%	34.7%
1-26c	8.9%	21.4%	32.4%	36.0%
1-8	8.9%	20.7%	31.9%	36.5%

CLAIMS

1. A method for producing xylitol, the method comprising:

contacting a substrate comprising dextrose in a fermentation broth with an engineered cell capable of producing xylitol, wherein fermentation of the substrate by the engineered cell produces xylitol, and

wherein during fermentation, a trehalase enzyme is present in the fermentation broth such that the concentration of trehalose at the end of fermentation is lower than an equivalent fermentation run without the trehalase enzyme.

2. The method of claim 1, wherein the trehalase enzyme is added to the fermentation broth exogenously.

3. The method of claim 1, wherein the engineered cell comprises an exogenous polynucleotide sequence encoding a secretion signal operably linked to a trehalase enzyme and the trehalase enzyme is excreted from the engineered cell.

4. A genetically engineered cell comprising an exogenous polynucleotide sequence encoding a secretion signal operably linked to a trehalase enzyme and the trehalase enzyme is excreted from the engineered cell.

5. The method or cell of claim 3 or 4, wherein the polypeptide sequence of the secretion signal is at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:29-32 and the polypeptide sequence of the trehalase enzyme is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of SEQ ID NOs:25-28, preferably SEQ ID NOs:25 or 26.

6. The method or cell of any one of claims 3-5, wherein the polynucleotide sequence encodes a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identical to at least one of 33, 35, 36, 38, 39, 40, and 43, preferably SEQ ID NOs:35, 36, 38, and 40, most preferably SEQ ID NOs:35, 36, and 40.

7. The method of any one of claims 1-3 or 5-6, wherein trehalose titer is reduced relative to an equivalent method lacking the trehalase enzyme, e.g., trehalose titer is reduced at least 25%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or 100% relative to an equivalent method lacking the trehalase enzyme.

8. The method of any one of claims 1-3 or 5-7, wherein the process does not produce any trehalose or trehalose titer is below detectable levels.

9. The method of any one of claims 1-3 or 5-8, wherein the fermentation temperature is at or between 25 °C to 45 °C, 30 °C to 40 °C, or 32 °C to 37 °C and the volumetric oxygen uptake rate (OUR) is between 5-80, 10-75, 15-70, 20-60, 30-50, or 40-50 mmol O_2/(L • h).

10. The method of any one of claims 1-3 or 5-9, wherein the xylitol is produced at a rate of at least 0.2, 0.3, 0.5, 0.75, or at least 1.0 g L^{-1} h^{-1}.

11. The method of any one of claims 1-3 or 5-10, wherein the xylitol titer is at least 20, 30, 50, 75, or 100 g/L when the fermentation is run at 35 °C for 96 hours.

12. The method of any one of claims 1-3 or 5-11, wherein the concentration of dextrose is at least 100 g/L.

13. The method or cell of any preceding claim, wherein the engineered cell comprises an exogenous polynucleotide sequence encoding a xylitol-phosphate dehydrogenase (XPDH) enzyme comprising a sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:5, 108-114, and 116, preferably at least one of SEQ ID NOs:5, 110, 111, and 114.

14. The method or cell of any preceding claim, wherein the engineered cell comprises an exogenous polynucleotide sequence encoding a xylulokinase (XKS) enzyme comprising a sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:103 and 104.

15. The method or cell of claim 14, wherein the engineered cell additionally comprises an exogenous polynucleotide sequence encoding a xylitol dehydrogenase (XDH) enzyme comprising a sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:117-119.

16. The method or cell of any preceding claim, wherein the cell is a yeast cell, for example an osmotolerant yeast cell.

17. The method or cell of claim 16, wherein the yeast cell is a cell of the subphylum Ustilaginomycotina, for example, a cell selected from the group consisting of *Trichosporonoides megachiliensis*, *Trychosporonoides oedocephalis*, *Trychosporonoides nigrescens*, *Pseudozyma tsukubaensis*, *Trigonopsis variabilis*, *Moniliella*, *Ustilaginomycetes*, *Trichosporon*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Penicillium*, *Torula*, *Pichia*, *Candida*, *Candida magnoliae*, and *Aureobasidium*.

18. The method or cell of any preceding claim, wherein the cell is a *Moniliella pollinis* cell.

19. The method or cell of any preceding claim, wherein the engineered cell comprises a genetic modification resulting in overexpression of a native enzyme with xylitol-5-phosphate phosphatase (X5PP) activity; and/or an exogenous polynucleotide sequence encoding an enzyme with xylitol-5-phosphate phosphatase (X5PP) activity.

20. The method or cell of claim 19, wherein the cell is a *Moniliella pollinis* cell and the genetic modification results in overexpression of a native X5PP enzyme with a sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:24, 101, 105, or 106, for example the genetic modification comprises addition of an exogenous polynucleotide sequence encoding the native X5PP enzyme such that the genetically engineered cell comprises at least one additional copy of a sequence encoding the native X5PP enzyme.

21. The method or cell of any preceding claim, wherein the yeast cell comprises an exogenous polynucleotide sequence encoding an enzyme with X5PP activity and a sequence at least 75%, at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:24 and 88-104, preferable at least one of SEQ ID NOs:24, 89-93, 97, and 99, most preferably SEQ ID NOs:24, 90, 91, 93, and 99.

22. The method or cell of any preceding claim, wherein the engineered cell is a *Moniliella pollinis* cell and the cell comprises a deletion or disruption of a native gene encoding an erythrose reductase enzyme at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to at least one of SEQ ID NOs:74 and 76.

23. The method of claim 22, wherein rate, titer, and/or yield of erythritol production is decreased relative to an equivalent fermentation run with an equivalent yeast cell in which the native gene encoding the erythrose reductase enzyme has not been deleted or disrupted.

24. The method claim 22 or 23, wherein the erythritol titer is less than 20, 18, 15, 12, 10, 5, 1, 0.5 g/L when the fermentation is run at 35 °C for 96 hours; wherein the erythritol production rate is less than 0.5, 0.4, 0.3, 0.25, 0.2, 0.15, or 0.1 g L^{-1} h^{-1}; and/or wherein the erythritol yield is less than 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1%.

25. Use of the engineered cell of any one of claims 4-22 to produce xylitol in a fermentation process with reduced trehalose.

FIG. 1

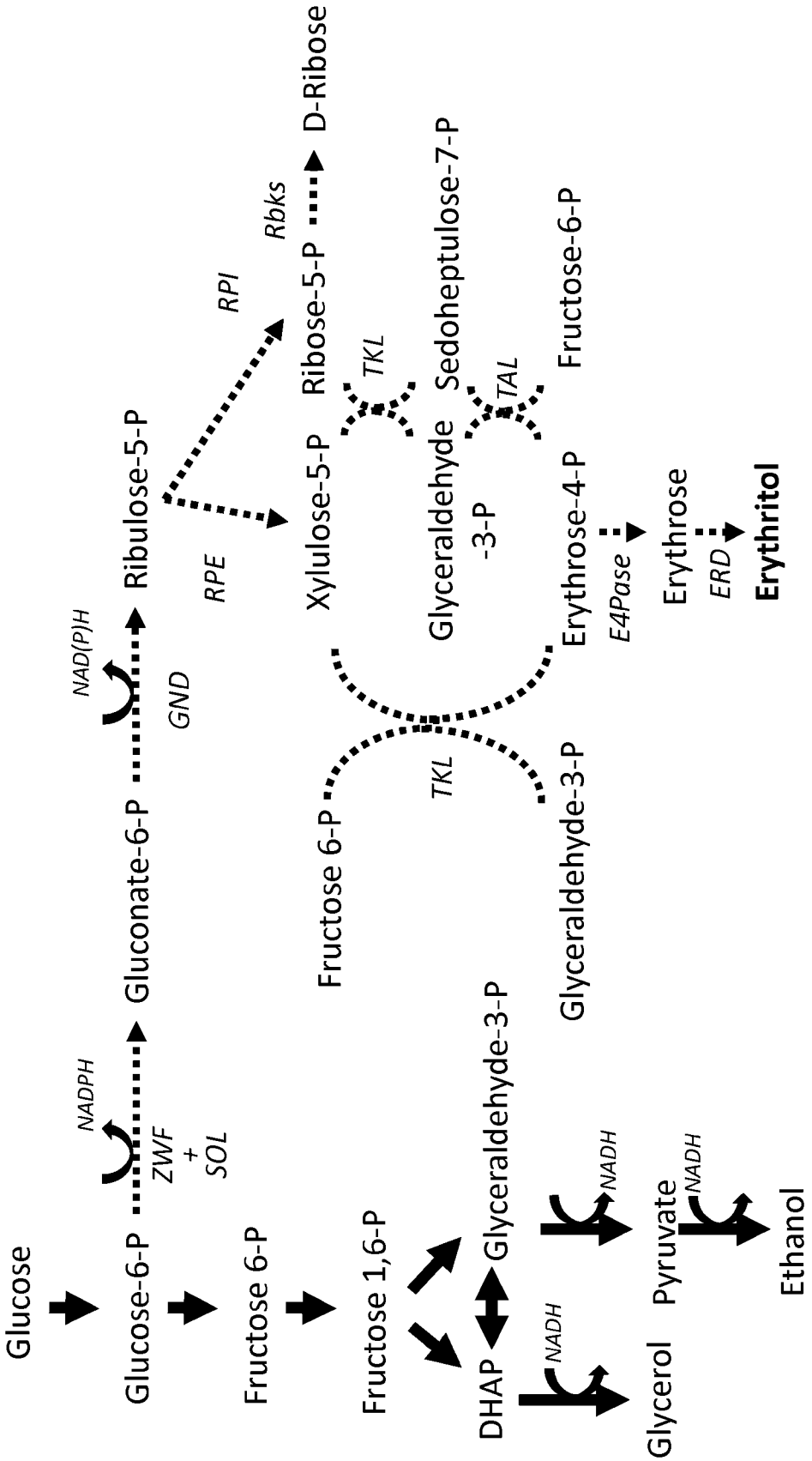

Glucose
Glucose-6-P
NADPH
ZWF
+
SOL
Fructose 6-P
Fructose 1,6-P
DHAP
Glyceraldehyde-3-P
NADH
Glycerol
Pyruvate
NADH
Ethanol
Gluconate-6-P
NAD(P)H
GND
Ribulose-5-P
RPE
RPI
Xylulose-5-P
Ribose-5-P
Rbks
D-Ribose
Fructose 6-P
TKL
Glyceraldehyde-3-P
TKL
Glyceraldehyde
-3-P
Sedoheptulose-7-P
TAL
Erythrose-4-P
Fructose-6-P
E4Pase
Erythrose
ERD
Erythritol

FIG. 2

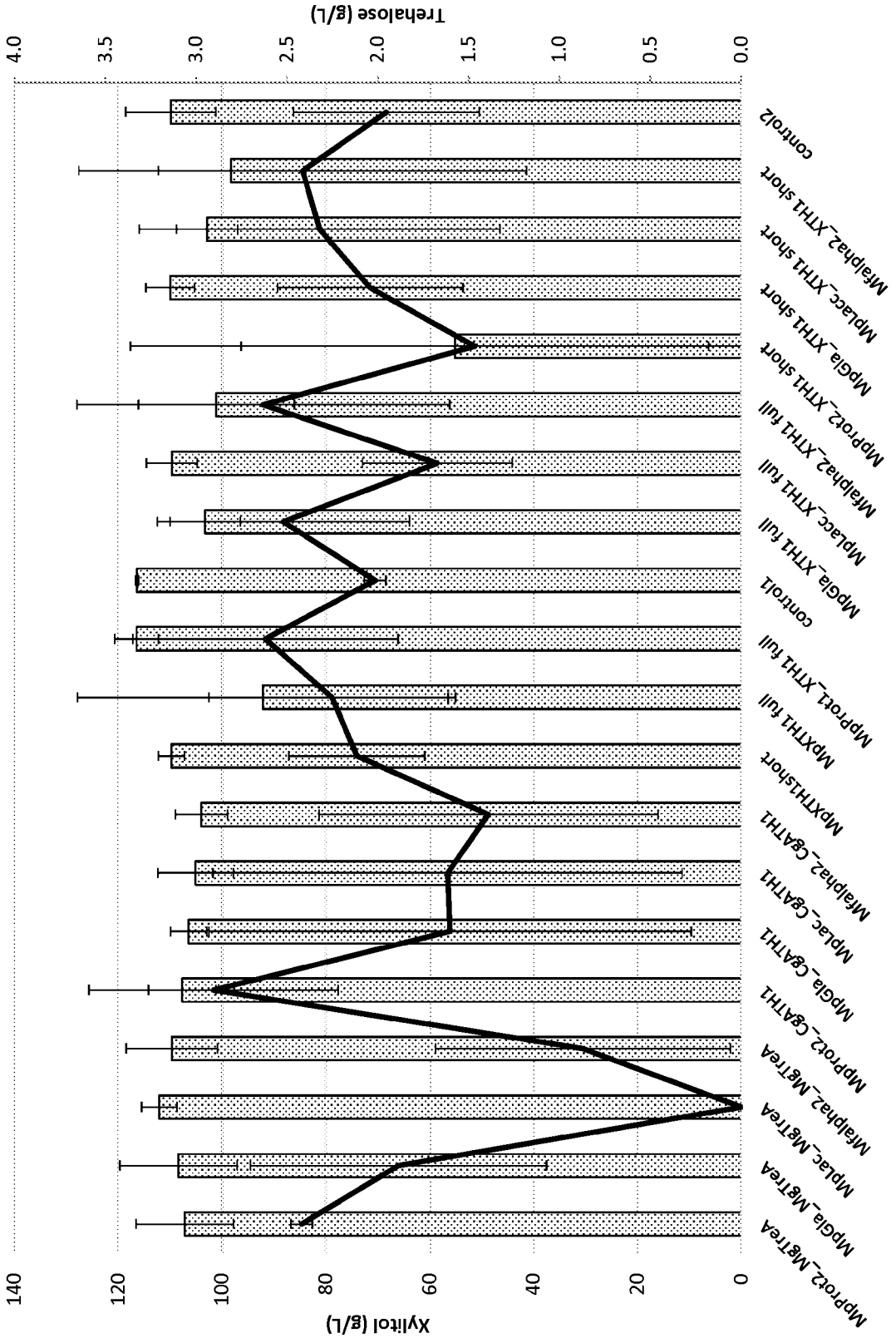
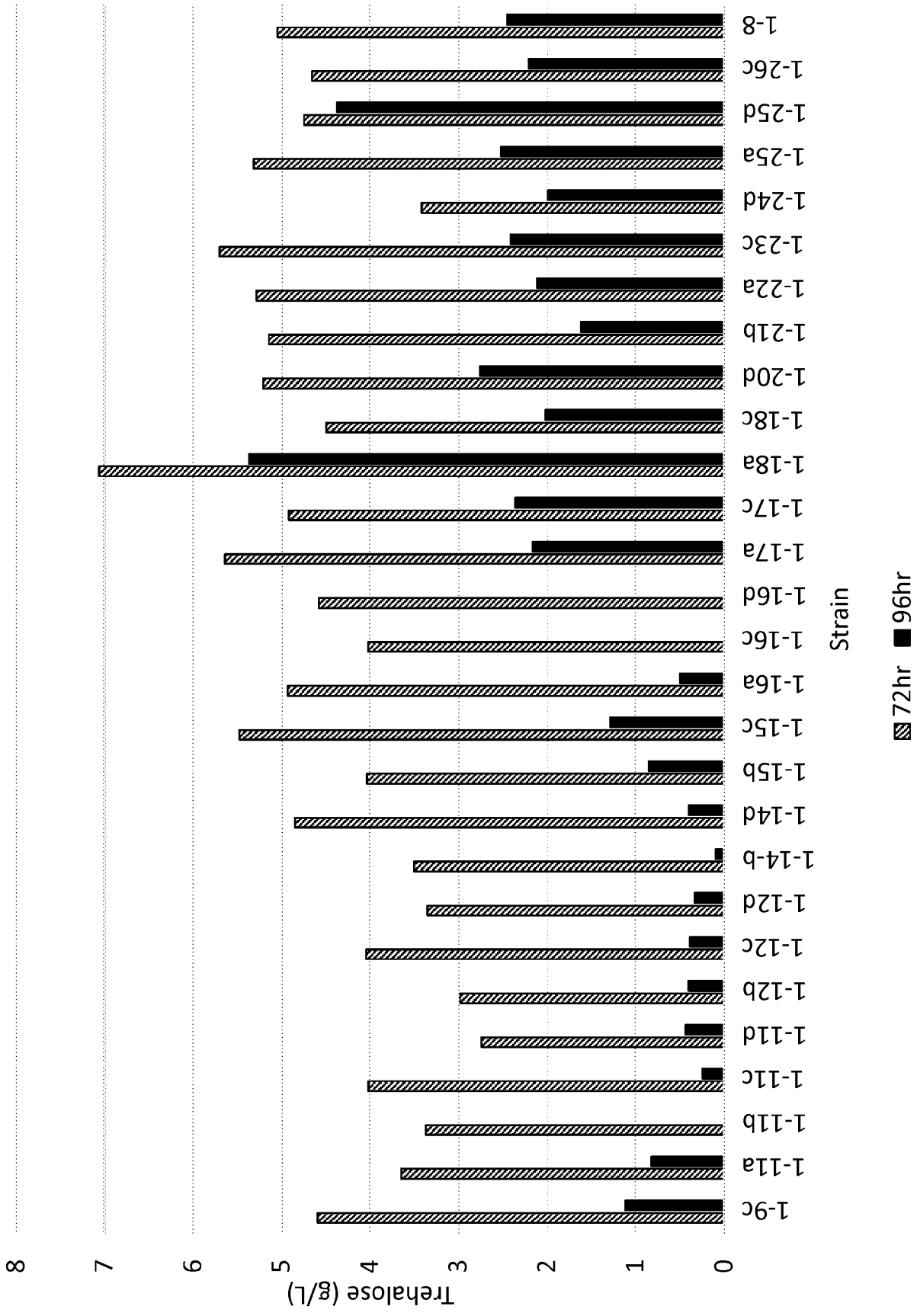

Xylitol (g/L)
0
20
40
60
80
100
120
140
Trehalose (g/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
MpProt2_MgTrea
MpGla_MgTrea
MpLac_MgTrea
MfalPha2_MgTrea
MpProt2_CgATH1
MpGla_CgATH1
MpLac_CgATH1
MfalPha2_CgATH1
MpXTH1short
MpXTH1 full
MpProt1_XTH1 full
control
MpGla_XTH1 full
MpLac_XTH1 full
MfalPha2_XTH1 full
MpProt2_XTH1 full
MpGla_XTH1 short
MpLac_XTH1 short
MfalPha2_XTH1 short
control2

FIG. 3

Erythritol
Xylitol
Polyols (g/L)
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
1-9c
1-11a
1-11b
1-11c
1-11d
1-12b
1-12c
1-12d
1-14b
1-14d
1-15b
1-15c
1-16a
1-16c
1-16d
1-17a
1-17c
1-18a
1-18c
1-20d
1-21b
1-22a
1-23c
1-24d
1-25a
1-25d
1-26c
1-8

FIG. 4


Trehalose (g/L)
0
1
2
3
4
5
6
7
8
Strain
72hr
96hr
1-9c
1-11a
1-11b
1-11c
1-11d
1-12b
1-12c
1-12d
1-14-b
1-14d
1-15b
1-15c
1-16a
1-16c
1-16d
1-17a
1-17c
1-18a
1-18c
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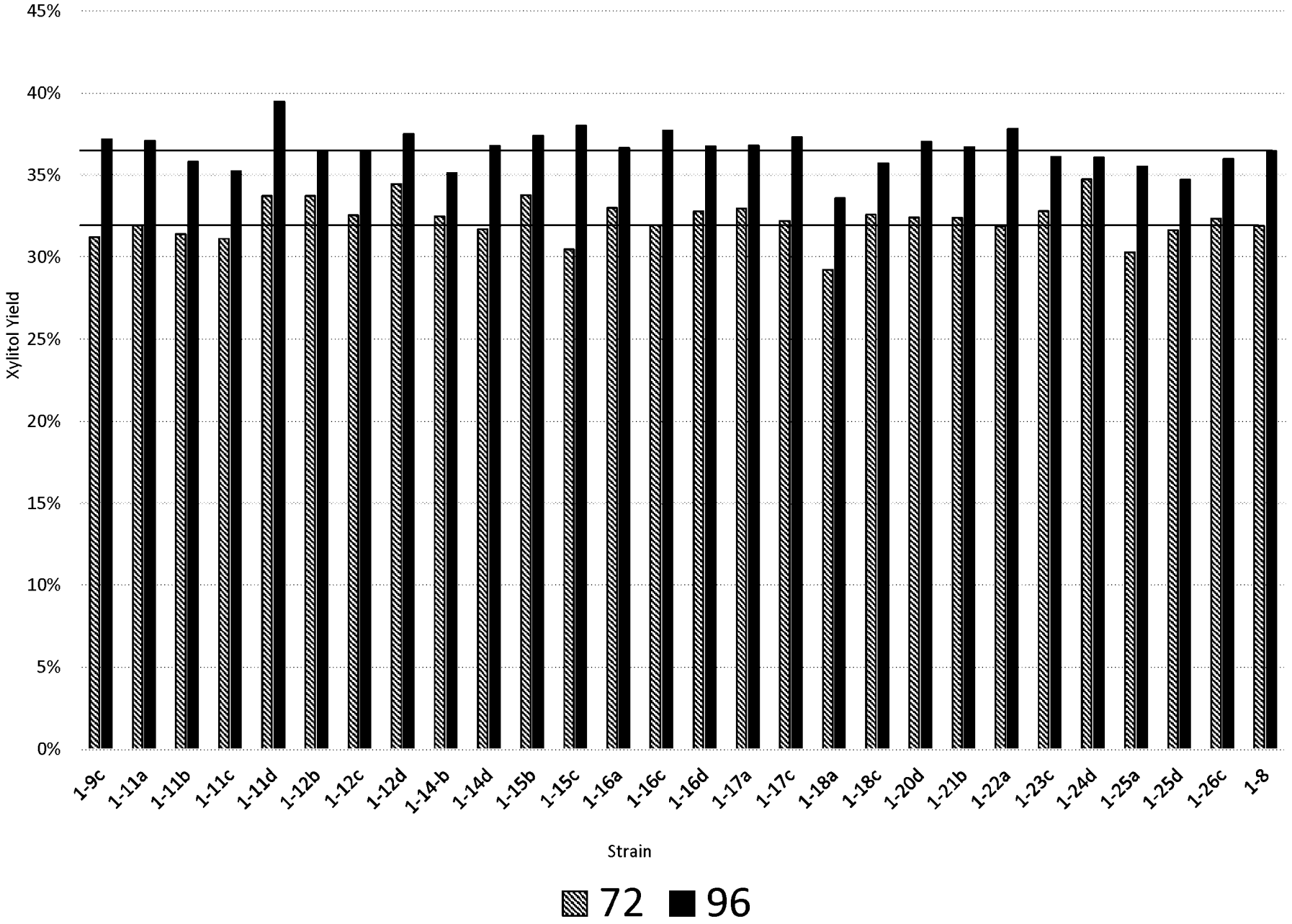
1-21b
1-22a
1-23c
1-24d
1-25a
1-25d
1-26c
1-8

FIG. 5


45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Xylitol Yield
1-9c
1-11a
1-11b
1-11c
1-11d
1-12b
1-12c
1-12d
1-14-b
1-14d
1-15b
1-15c
1-16a
1-16c
1-16d
1-17a
1-17c
1-18a
1-18c
1-20d
1-21b
1-22a
1-23c
1-24d
1-25a
1-25d
1-26c
1-8
Strain
72
96