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(54) Titre : PROCEDE ET ORGANISME EXPRIMANT DES TRANSPORTEURS DE XYLOSE DE METSCHNIKOWIA POUR UNE ABSORPTION ACCRUE DU XYLOSE
(54) Title: METHOD AND ORGANISM EXPRESSING METSCHNIKOWIA XYLOSE TRANSPORTERS FOR INCREASED XYLOSE UPTAKE

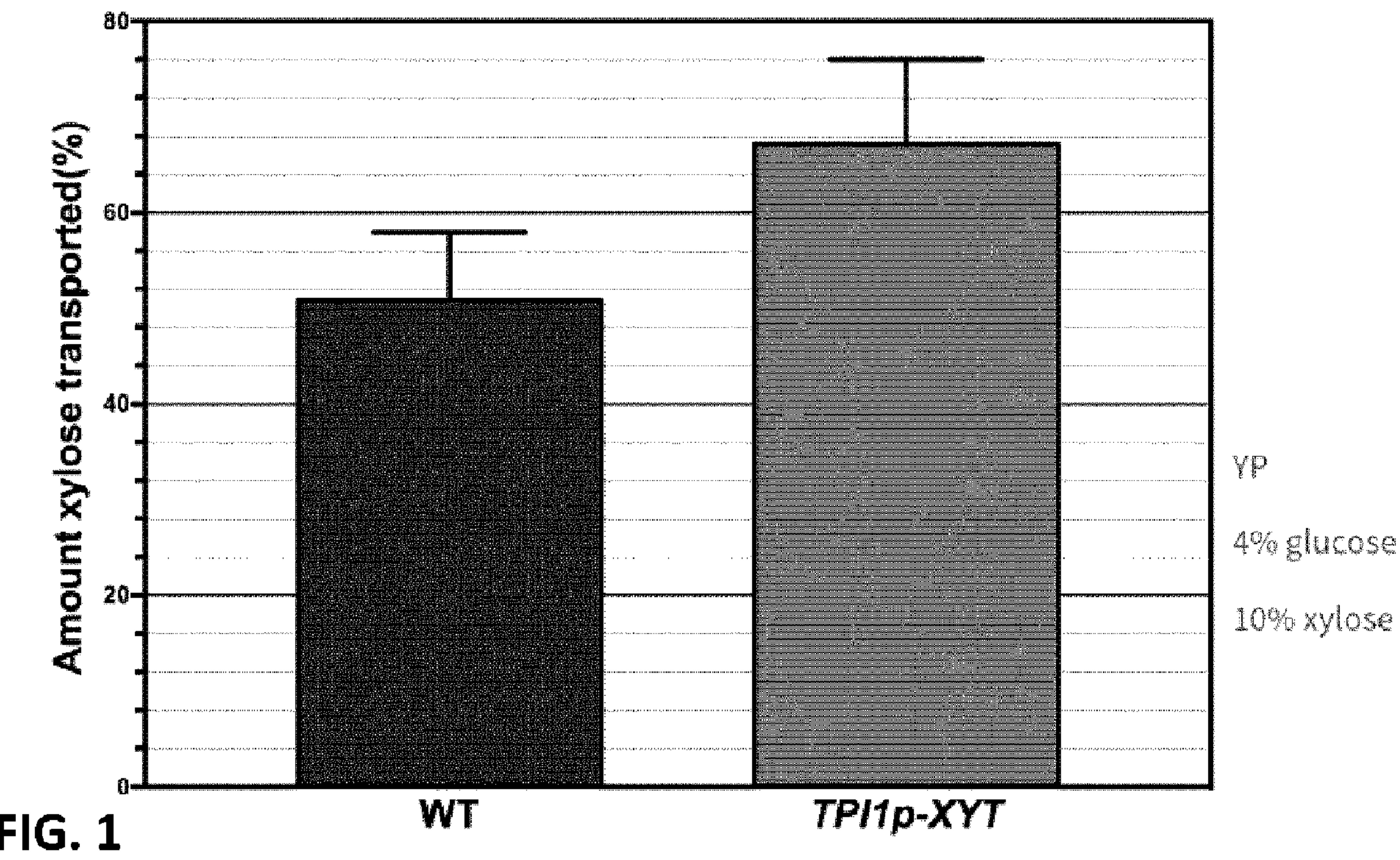

FIG. 1

(57) Abrégé/Abstract:
Provided are novel xylose transporters and genetically modified transporters derived from a Metschnikowia strain having Accession Number 081116-01, expressed in Metschnikowia strain Accession No. 081116-01 and Saccharomyces cerevisiae, and methods

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of use to produce bioderived compounds, including xylitol, from xylose.

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(54) Title: METHOD AND ORGANISM EXPRESSING *METSCHNIKOWIA* XYLOSE TRANSPORTERS FOR INCREASED XYLOSE UPTAKE

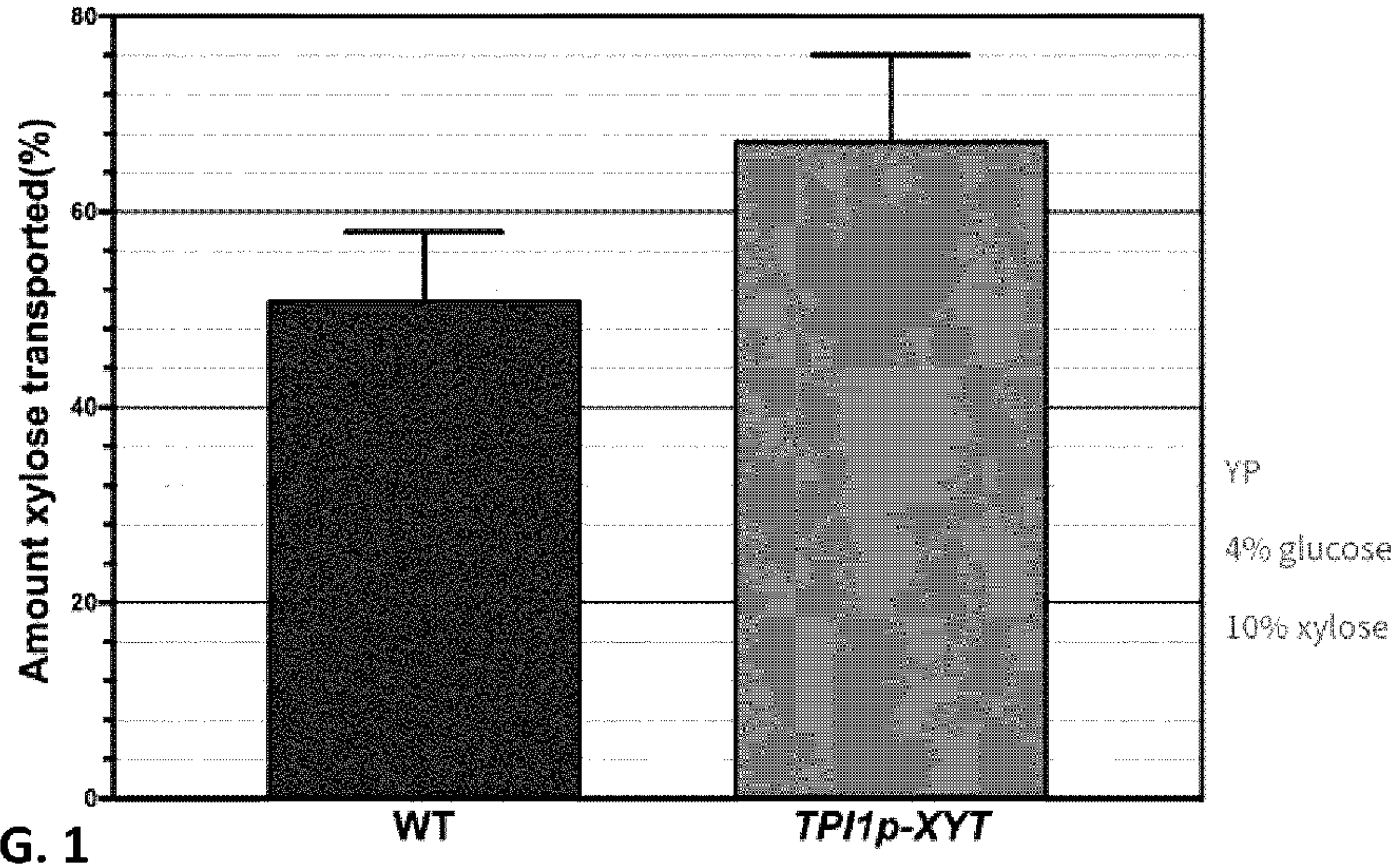

FIG. 1

(57) Abstract: Provided are novel xylose transporters and genetically modified transporters derived from a *Metschnikowia* strain having Accession Number 081116-01, expressed in *Metschnikowia* strain Accession No. 081116-01 and *Saccharomycescerevisiae*, and methods of use to produce bioderived compounds, including xylitol, from xylose.

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METHOD AND ORGANISM EXPRESSING METSCHNIKOWIA XYLOSE TRANSPORTERS FOR INCREASED XYLOSE UPTAKE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of United States Provisional Application No. 62/437,600, filed on December 21, 2016, the content of which is herein
5 incorporated by reference in its entirety.

FIELD

[0002] The present invention relates to the field of molecular biology and microbiology. Provided herein are non-naturally occurring microbial organisms having increased xylose uptake and increased production of bioderived compounds using xylose as a substrate, as well
10 as methods to make and use these microbial organisms.

REFERENCE TO SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 19, 2017, is named 14305-007-228_Sequence_Listing.txt
15 and is 146,836 bytes in size.

BACKGROUND

[0004] Xylose is an abundant sugar present in lignocellulosic biomass, a renewable feedstock for producing bioderived chemicals. However, the use of lignocellulosic biomass and the production of bioderived chemicals are limited by the naturally low xylose uptake in
20 microbial organisms. Therefore, methods to increase xylose uptake in microbial organisms to increase the production of bioderived compounds from xylose represent unmet needs. The non-naturally occurring microbial organisms and methods provided herein meet these needs and provide other related advantages.

25 ## SUMMARY OF THE INVENTION

[0005] Provided herein are non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 89% identical to a *Metschnikowia* xylose transporter.

[0006] In some embodiments, the non-naturally occurring microbial organism provided herein can have exogenous nucleic acids encoding at least two, at least three, at least four, at least five, at least six, or at least seven xylose transporters. In some embodiments, the xylose transporter can have an amino acid sequence that is at least 90%, at least 95%, at least 98%, or at least 99%, identical to the *Metschnikowia* xylose transporter. In some embodiments, the xylose transporter is a *Metschnikowia* xylose transporter. In some embodiments, the *Metschnikowia* xylose transporter can be, for example, Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

[0007] In some embodiments, the *Metschnikowia* xylose transporter is from the *H0 Metschnikowia sp.* Accordingly, also provided herein is a non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 89% identical to a xylose transporter form the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 89% identical to SEQ ID NO: 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, or 12. In some embodiments, the non-naturally occurring microbial organism provided herein can have the exogenous nucleic acid SEQ ID NOs: 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24 25, 26, or 27.

[0008] In some embodiments, the xylose transporter can be ubiquitin-deficient. In some embodiments, the xylose transporter has an amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 45. In some embodiments, the non-naturally occurring microbial organism provided herein has the exogenous nucleic acid SEQ ID NO: 49 or SEQ ID NO: 45.

[0009] In some embodiments, the exogenous nucleic acid can be codon-optimized for expression in the host microbial organism.

[0010] In some embodiments, provided herein is a non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 74% identical to Xyt1p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 74% identical to SEQ ID NO: 1. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 13 or SEQ ID NO: 21.

[0011] In some embodiments, provided herein is a non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 85% identical to Gxf1p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 74% identical to SEQ ID NO: 2. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 14.

[0012] In some embodiments, provided herein is a non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 89% identical to a ΔGxf1p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 89% identical to SEQ ID NO: 3. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 15.

[0013] In some embodiments, provided herein is a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 71% identical to a Gxf2p/Gal2p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 71% identical to SEQ ID NO: 4. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 16.

[0014] In some embodiments, provided herein is a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 71% identical to a Gxs1p/Hgt12p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 71% identical to SEQ ID NO: 7. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 19.

[0015] In some embodiments, provided herein is a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 60% identical to a

Hxt5p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 60% identical to SEQ ID NO: 8. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 20.

5 **[0016]** In some embodiments, provided herein is a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 84% identical to a Hxt2.6p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid
10 sequence that is at least 84% identical to SEQ ID NO: 10. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 22 or SEQ ID NO: 23.

[0017] In some embodiments, provided herein is a non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 50% identical to a
15 Qup2p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 50% identical to SEQ ID NO: 11. In some embodiments, the exogenous nucleic acid has the sequence of SEQ ID NO: 24 or SEQ ID NO: 25.

[0018] In some embodiments, provided herein is a non-naturally occurring microbial
20 organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 74% identical to a Aps1p/Hgt19p of a *Metschnikowia* species. In some embodiments, the *Metschnikowia* species is the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has an amino acid sequence that is at least 74% identical to SEQ ID NO: 12. In some embodiments,
25 the exogenous nucleic acid has the sequence of SEQ ID NO: 26 or SEQ ID NO: 27.

[0019] Provided herein are non-naturally occurring microbial organism having at least one exogenous nucleic acid encoding a xylose transporter having an amino acid sequence that is at least 89% identical to a *Metschnikowia* xylose transporter. The exogenous nucleic acid can be a heterologous nucleic acid. The microbial organism can be in an aerobic culture
30 medium or a substantially anaerobic culture medium. The microbial organism can be a species of bacteria or yeast.

[0020] In some embodiments, the microbial organism is a species of a yeast, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Candida tropicalis*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Aspergillus terreus*, *Aspergillus niger*, *Chlamydomonas reinhardtii*, *Pichia pastoris*,
5 *Rhizopus arrhizus*, *Rhizobus oryzae*, *Trichoderma reesei*, or *Yarrowia lipolytica.*

[0021] In some embodiments, the microbial organism is a species of a bacteria, such as *Escherichia coli*, *Klebsiella oxytoca*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Rhizobium etli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Gluconobacter oxydans*, *Zymomonas mobilis*, *Lactococcus*
10 *lactis*, *Lactobacillus plantarum*, *Streptomyces coelicolor*, *Clostridium acetobutylicum*, *Pseudomonas fluorescens*, or *Pseudomonas putida*.

[0022] The non-naturally occurring microbial organism provided herein can further include a metabolic pathway capable of producing a bioderived compound from xylose, such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl
15 alcohol, 2-methyl-butanol, or 3-methyl-butanol.

[0023] Provided herein is also a method of producing a bioderived compound, including culturing the non-naturally occurring microbial organism provided herein under conditions and for a sufficient period of time to produce said bioderived compound, wherein the microbial organism has a pathway capable of producing the bioderived compound from
20 xylose. The microbial organism can be cultured in medium having xylose and a co-substrate, such as cellobiose, hemicellulose, glycerol, galactose, and glucose, or a combination thereof. The microbial organism can be cultured in batch cultivation, fed-batch cultivation or continuous cultivation.

[0024] In some embodiments, the method can further includes separating the bioderived
25 compound from other components in the culture. The separation method can include, for example, extraction, continuous liquid-liquid extraction, pervaporation, membrane filtration, membrane separation, reverse osmosis, electrodialysis, distillation, crystallization, centrifugation, extractive filtration, ion exchange chromatography, absorption chromatography, or ultrafiltration.

30 [0025] Also provided herein is a bioderived compound produced by the method described herein. The bioderived compound can include, for example, glycerol, acetaldehyde, acetate,

glyceraldehyde, or a combination thereof as impurities. Also provided herein is a composition having one or more of the bioderived compound described herein. In some embodiments, the composition can have the bioderived xylitol. In some embodiments, the composition can be culture medium. The composition can be culture medium with the
5 microbial organism removed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1 shows the efficient xylose uptake by the wild type *H0 Metschnikowia sp.* measured by the amount of xylose transported (%), which was further enhanced (from about 55% to about 65%) when *XYT1*, a xylose transporter of the *H0 Metschnikowia sp.*, was
10 overexpressed.

[0027] FIG. 2 shows the efficient xylose uptake by the wild type *H0 Metschnikowia sp.* measured by xylose transported (%) per unit OD_{600}, which was further enhanced (from about 1.3 to 2.2) when the *XYT1* was overexpressed.

[0028] FIG. 3 shows that the expression of H0 *XYT1* in *Saccharomyces* increased the
15 xylose transport from about 10% to about 74% (48 hours) in *Saccharomyces*.

[0029] FIGs. 4A-4C show the xylose uptake by host strain BY4742 (*Saccharomyces cerevisiae*), and BY4742 strains expressing xylose transports *H0 Metschnikowia sp.* Gxf2p/Gal2p ("Gal2p"), *H0 Metschnikowia sp.* Gxf1p ("Gxf1p"), *H0 Metschnikowia sp.* Xyt1p ("Xyt1p"), *H0 Metschnikowia sp.* Hxt5p ("Hxt5p"), *H0 Metschnikowia sp.*
20 Aps1p/Hgt19p ("Hgt19p"), *Candida intermedia* Gxf1p ("CiGxf1p-65d"), *Pichia stipis* Sut1p ("PsSut1p"), ubiquitin-deficient *H0 Metschnikowia sp.* Aps1p/Hgt19p ("Δubq-Hgt19p"), or ubiquitin-deficient *H0 Metschnikowia sp.* Hxt5p ("Δubq- Hxt5p"), at 18h (FIG. 4A), 64h (FIG. 4B), and 88h (FIG. 4C)

DETAILED DESCRIPTION

25 **[0030]** The compositions and methods provided herein are based, in part, on the discovery, cloning and characterization of novel xylose transporters from the *Metschnikowia* genus. Expression of one or more of these xylose transporters or variants thereof in host microbial organisms was found to increase xylose uptake, as well as production of bioderived compounds by these microbial organisms using xylose as a substrate. Provided herein are

also nucleic acids that encode these xylose transporters, non-naturally microbial organisms having enhanced xylose uptake by expressing these xylose transporters, as well as bioderived compounds produced by these microbial organisms.

[0031] As used herein, the term “non-naturally occurring,” when used in reference to a microbial organism or microorganism described herein is intended to mean that the microbial organism has at least one genetic alteration not normally found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Genetic alterations include, for example, modifications introducing expressible nucleic acids encoding metabolic polypeptides, other nucleic acid additions, nucleic acid deletions and/or other functional disruption of the microbial organism’s genetic material. Such modifications include, for example, coding regions and functional fragments thereof, for heterologous, homologous or both heterologous and homologous polypeptides for the referenced species. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a gene or operon. Exemplary metabolic polypeptides include, but are not limited to, enzymes or proteins within a xylitol biosynthetic pathway.

[0032] As used herein, the terms “microbial,” “microbial organism” or “microorganism” are intended to mean any organism that exists as a microscopic cell that is included within the domains of archaea, bacteria or eukarya. Therefore, the term is intended to encompass prokaryotic or eukaryotic cells or organisms having a microscopic size and includes bacteria, archaea and eubacteria of all species as well as eukaryotic microorganisms such as yeast and fungi. The term also includes cell cultures of any species that can be cultured for the production of a biochemical.

[0033] As used herein, the term “isolated” when used in reference to a microbial organism is intended to mean an organism that is substantially free of at least one component as the referenced microbial organism is found in nature. The term includes a microbial organism that is removed from some or all components as it is found in its natural environment. The term also includes a microbial organism that is removed from some or all components as the microbial organism is found in non-naturally occurring environments. Therefore, an isolated microbial organism is partly or completely separated from other substances as it is found in nature or as it is grown, stored or subsisted in non-naturally occurring environments. Specific examples of isolated microbial organisms include partially

pure microbes, substantially pure microbes and microbes cultured in a medium that is non-naturally occurring.

[0034] As used herein, the terms "exogenous" is intended to mean that the referenced molecule or the referenced activity is introduced into the host microbial organism. The molecule can be introduced, for example, by introduction of an encoding nucleic acid into the host genetic material such as by integration into a host chromosome or as non-chromosomal genetic material such as a plasmid. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the microbial organism. When used in reference to a biosynthetic activity, the term refers to an activity that is introduced into the host reference organism. The source can be, for example, a homologous or heterologous encoding nucleic acid that expresses the referenced activity following introduction into the host microbial organism. Therefore, the term "endogenous" refers to a referenced molecule or activity that is present in the host. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid contained within the microbial organism. The term "heterologous" refers to a molecule or activity derived from a source other than the referenced species whereas "homologous" refers to a molecule or activity derived from the host microbial organism. Accordingly, exogenous expression of an encoding nucleic acid of the invention can utilize either or both a heterologous or homologous encoding nucleic acid.

[0035] It is understood that when more than one exogenous nucleic acid is included in a microbial organism that the more than one exogenous nucleic acids refers to the referenced encoding nucleic acid or biosynthetic activity, as discussed above. It is also understood that a microbial organism can have one or multiple copies of the same exogenous nucleic acid. It is further understood, as disclosed herein, that such more than one exogenous nucleic acids can be introduced into the host microbial organism on separate nucleic acid molecules, on polycistronic nucleic acid molecules, or a combination thereof, and still be considered as more than one exogenous nucleic acid. For example, as disclosed herein a microbial organism can be engineered to express two or more exogenous nucleic acids encoding a desired pathway enzyme or protein. In the case where two exogenous nucleic acids encoding a desired activity are introduced into a host microbial organism, it is understood that the two exogenous nucleic acids can be introduced as a single nucleic acid, for example, on a single

plasmid, on separate plasmids, can be integrated into the host chromosome at a single site or multiple sites, and still be considered as two exogenous nucleic acids. Similarly, it is understood that more than two exogenous nucleic acids can be introduced into a host organism in any desired combination, for example, on a single plasmid, on separate plasmids, can be integrated into the host chromosome at a single site or multiple sites, and still be considered as two or more exogenous nucleic acids, for example three exogenous nucleic acids. Thus, the number of referenced exogenous nucleic acids or biosynthetic activities refers to the number of encoding nucleic acids or the number of biosynthetic activities, not the number of separate nucleic acids introduced into the host organism.

[0036] As used herein, the term "xylose" refers to a five carbon monosaccharide with a formyl functional group having the chemical formula of $C_5H_{10}O_5$, a Molar mass of 150.13 g/mol, and one IUPAC name of (3*R*,4*S*,5*R*)-oxane-2,3,4,5-tetrol. Xylose is also known in the art as D-xylose, D-xylopyranose, xyloside, d-(+)-xylose, xylopyranose, wood sugar, xylomed and D-xylopentose.

[0037] As used herein, the term "xylose transporter" refers to membrane protein that facilitates the movement of xylose across a cell membrane. The term "*Metschnikowia* xylose transporter" refers to a xylose transporter from a *Metschnikowia* species. As used herein, the term "*Metschnikowia* species" refers to any species of yeast that falls within the *Metschnikowia* genus. Exemplary *Metschnikowia* species include, but are not limited to, *Metschnikowia pulcherrima, Metschnikowia fructicola, Metschnikowia chrysoperlae, Metschnikowia andauensis, Metschnikowia shanxiensis, Metschnikowia sinensis, Metschnikowia zizyphicola, Metschnikowia reukaufii, Metschnikowia bicuspidata, Metschnikowia lunata, Metschnikowia zobellii, Metschnikowia australis, Metschnikowia agaveae, Metschnikowia gruessii, Metschnikowia hawaiiensis, Metschnikowia krissii, Metschnikowia sp. strain NS-O-85, Metschnikowia sp. strain NS-O-89, Metschnikowia sp. strain 4MS-2013* and the unique *Metschnikowia* species described herein, *Metschnikowia sp. H0*, alternatively known as the "*H0 Metschnikowia sp.*" The *Metschnikowia* species described herein, *i.e.*, the *H0 Metschnikowia sp.*, is a newly discovered species, which is identified by the designated Accession No. 081116-01, and deposited at International Depositary Authority of Canada ("IDAC"), an International Depositary Authority, at the address of 1015 Arlington Street, Winnipeg, Manitoba, Canada R3E 3R2, on November 8, 2016, under the terms of the Budapest Treaty.

[0038] As used herein, the term "ubiquitin-deficient" when used in connection with a protein refers to an altered form of the protein that is resistant to ubiquitination at one or more ubiquitination sites and proteasome-mediated degradation. The resistance to ubiquitination can range from a decreased frequency of ubiquitination to complete inhibition of ubiquitination. Ubiquitination is an enzymatic post-translational modification by which a ubiquitin protein is attached to a lysine residue of the substrate protein. A chain of multiple ubiquitin proteins can form on a single lysine residue on the substrate protein, and target the substrate protein for proteasome-mediated degradation. Accordingly, a ubiquitin-deficient protein is partially or totally resistant to ubiquitination and proteasome-mediated degradation. In some embodiments, a ubiquitin-deficient protein has an amino acid mutation at or near a lysine residue that can be ubiquitinated. The proximity of the mutation near a lysine residue that can be ubiquitinated can be close within the primary sequence or close within the 3D structure so long as the mutation yields resistance to ubiquitination. Such mutation can be amino acid substitution, deletion, or addition. In some embodiment, the lysine residue that can be ubiquitinated itself is substituted to another amino acid. In some embodiment, the lysine residue is deleted. In some embodiments, one or more amino acid residues near the lysine residue that can be ubiquitinated are mutated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, a ubiquitin-deficient protein has amino acid mutations at or near at least two lysine residues that can be ubiquitinated. In some embodiments, a ubiquitin-deficient protein has amino acid mutations at or near at least three lysine residues that can be ubiquitinated. In some embodiments, a ubiquitin-deficient protein has amino acid mutations at or near at least four lysine residues that can be ubiquitinated. In some embodiments, a ubiquitin-deficient protein has amino acid substitutions at or near all lysine residues that can be ubiquitinated. In some embodiments, a ubiquitin-deficient protein has amino acid substitutions at least all lysine residues that can be ubiquitinated, and is completely resistant to ubiquitination and proteasome-mediated degradation.

[0039] As used herein, the term "medium," "culture medium," "growth medium" or grammatical equivalents thereof refers to a liquid or solid (*e.g.*, gelatinous) substance containing nutrients that supports the growth of a cell, including any microbial organism species described herein. Nutrients that support growth include: a substrate that supplies carbon, such as, but are not limited to, xylose, cellobiose, hemicelluloses, glycerol, galactose and glucose; salts that provide essential elements including magnesium, nitrogen,

phosphorus, and sulfur; a source for amino acids, such as peptone or tryptone; and a source for vitamin content, such as yeast extract. Specific examples of medium useful in the methods and in characterizing the *Metschnikowia* species described herein include yeast extract peptone (YEP) medium and yeast nitrogen base (YNB) medium having a carbon source such as, but not limited to xylose, glucose, cellobiose, galactose, or glycerol, or a combination thereof. The formulations of YEP and YNB medium are well known in the art. For example, YEP medium having 4% xylose includes, but is not limited to, yeast extract 1.0 g, peptone 2.0 g, xylose 4.0 g, and 100 ml water. As another example, YNB medium having 2% glucose and 2% xylose includes, but is not limited to, biotin 2 µg, calcium pantothenate 400 µg, folic acid 2 µg, inositol 2000 µg, niacin 400 µg, paminobenzoic acid 200 µg, pyridoxine hydrochloride 400 µg, riboflavin 200 µg, thiamine hydrochloride 400 µg, boric acid 500 µg, copper sulfate 40 µg, potassium iodide 100 µg, ferric chloride 200 µg, manganese sulfate 400 µg, sodium molybdate 200 µg, zinc sulfate 400 µg, potassium phosphate monobasic 1 g, magnesium sulfate 500 mg, sodium chloride 100 mg, calcium chloride 100 mg, 20 g glucose, 20 g, xylose and 1 L water. The amount of the carbon source in the medium can be readily determined by a person skilled in the art. When more than one substrate that supplies carbon is present in the medium, these are referred to as "co-substrates." Medium can also include substances other than nutrients needed for growth, such as a substance that only allows select cells to grow (*e.g.*, antibiotic or antifungal), which are generally found in selective medium, or a substance that allows for differentiation of one microbial organism over another when grown on the same medium, which are generally found in differential or indicator medium. Such substances are well known to a person skilled in the art.

[0040] As used herein, the term "aerobic" when used in reference to a culture or growth condition is intended to mean that the free oxygen (O_2) is available in the culture or growth condition. The term "anaerobic" when used in reference to a culture or growth condition is intended to mean that the culture or growth condition lacks free oxygen (O_2). The term "substantially anaerobic" when used in reference to a culture or growth condition is intended to mean that the amount of dissolved oxygen in a liquid medium is less than about 10% of saturation. The term also is intended to include sealed chambers maintained with an atmosphere of less than about 1% oxygen that include liquid or solid medium.

[0041] As used herein, the term “bioderived” means derived from or synthesized by a biological organism and can be considered a renewable resource since it can be generated by a biological organism. Such a biological organism, in particular the microbial organism disclosed herein, can utilize feedstock or biomass, such as, sugars (*e.g.*, xylose, glucose, 5 fructose, galactose, sucrose, and arabinose), carbohydrates obtained from an agricultural, plant, bacterial, or animal source, and glycerol.

[0042] As used herein, the term "biobased" means a product is composed, in whole or in part, of a bioderived compound. A biobased or bioderived product is in contrast to a petroleum derived product, wherein such a product is derived from or synthesized from 10 petroleum or a petrochemical feedstock.

[0043] Provided herein are novel *Metschnikowia* xylose transporters. Expression of these transporters or their variants in microbial organisms (*e.g. Saccharomyces cerevisiae*) can enhance xylose uptake and increase the production of bioderived products from xylose by these microbial organisms. Thus, provided herein is an isolated polypeptide that is a 15 *Metschnikowia* xylose transporter or a variant thereof; an isolated nucleic acid that encodes a *Metschnikowia* xylose transporter or a variant thereof; a vector that has an isolated nucleic acid that encodes a *Metschnikowia* xylose transporter or a variant thereof; as well as a non-naturally occurring microbial organism having enhanced xylose uptake and at least one exogenous nucleic acid encoding a *Metschnikowia* xylose transporter or a variant thereof.

20 **[0044]** Provided herein are non-naturally occurring microbial organisms having enhanced xylose uptake, which have at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30% identical to a *Metschnikowia* xylose transporter. The microbial organisms can have one or more copies of the exogenous nucleic acid. In some embodiments, the microbial organisms can have two, 25 three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more copies of the exogenous nucleic acid.

[0045] Provided herein are also isolated polypeptides that are at least 30% identical to a *Metschnikowia* xylose transporter. Provided herein are also isolated nucleic acids that encode polypeptides that are at least 30% identical to a *Metschnikowia* xylose transporter. The 30 *Metschnikowia* xylose transporters include, for example, transporters such as Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or

Aps1p/Hgt19p from a *Metschnikowia* species. The *Metschnikowia* species include, for example, the *Metschnikowia sp. H0*, *Metschnikowia pulcherrima, Metschnikowia fructicola, Metschnikowia chrysoperlae, Metschnikowia andauensis, Metschnikowia shanxiensis, Metschnikowia sinensis, Metschnikowia zizyphicola, Metschnikowia reukaufii,*
5 *Metschnikowia bicuspidata, Metschnikowia lunata, Metschnikowia zobellii, Metschnikowia australis, Metschnikowia agaveae, Metschnikowia gruessii, Metschnikowia hawaiiensis, Metschnikowia krissii, Metschnikowia sp. strain NS-O-85, Metschnikowia sp. strain NS-O-89,* and *Metschnikowia sp. strain 4MS-2013.* The *Metschnikowia* xylose transporter can be a xylose transporter from the *H0 Metschnikowia sp.* In some embodiments, the xylose
10 transporter can include, for example, Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from the *H0 Metschnikowia sp.* Exemplary sequences are provided below.

SEQ ID NO:	Description	SEQUENCES
1	Amino acid sequence of *H0 Metschnikowia species* Xyt1p	MGYEEKLVAPALKFKNFLDKTPNIHNVYVIAAISCTSGMMFGFDISSMSVF VDQQPYLKMFDNPSSVIQGFITASMSLGSFFGSLTSTFISEPFGRRASLFICGI LWVIGAAVQSSSQNRAQLICGRIIAGWGIGFGSSVAPVYGSEMAPRKIRGTI GGIFQFSVTVGIFIMFLIGYGCSFIQGKASFRIPWGVQMVPGLILLIGLFFIPES PRWLAKQGYWEDAEIIVANVQAKGNRNDANVQIEMSEIKDQLMLDEHLK EFTYADLFTKKYRQRTITAIFAQIWQQLTGMNVMMYYIVYIFQMAGYSGN TNLVPSLIQYIINMAVTVPALFCLDLLGRRTILLAGAAFMMAWQFGVAGIL ATYSEPAYISDTVRITIPDDHKSAAKGVIACCYLFVCSFAFSWGVGIWVYCS EVWGDSQSRQRGAALATSANWIFNFAIAMFTPSSFKNITWKTYIIYATFCAC MFIHVFFFFPETKGKRLEEIGQLWDEGVPAWRSAKWQPTVPLASDAELAH KMDVAHAEHADLLATHSPSSDEKTGTV
2	Amino acid sequence of *H0 Metschnikowia species* Gxf1p	MSQDELHTKSGVETPINDSLLEEKHDVTPLAALPEKSFKDYISISIFCLFVAF GGFVFGFDTGTISGFVNMSDFKTRFGEMNAQGEYYLSNVRTGLMVSIFNV GCAVGGIFLCKIADVYGRRIGLMFSMVVYVVGIIIQIASTTKWYQYFIGRLI AGLAVGTVSVISPLFISEVAPKQLRGTLVCCFQLCITLGIFLGYCTTYGTKTY TDSRQWRIPLGICFAWALFLVAGMLNMPESPRYLVEKSRIDDARKSIARSN KVSEEDPAVYTEVQLIQAGIDREALAGSATWMELVTGKPKIFRRVIMGVM LQSLQQLTGDNYFFYYGTTIFKAVGLQDSFQTSIILGIVNFASTFVGIYAIER MGRRLCLLTGSACMFVCFIIYSLIGTQHLYKNGFSNEPSNTYKPSGNAMIFIT CLYIFFFASTWAGGVYCIVSESYPLRIRSKAMSVATAANWMWGFLISFFTPF ITSAIHFYYGFVFTGCLAFSFFYVYFFVVETKGLSLEEVDILYASGTLPWKSS GWVP
3	Amino acid sequence of *H0 Metschnikowia species* ΔGxf1p (variant of Gxf1p with shorter N-terminus)	MSDFKTRFGEMNAQGEYYLSNVRTGLMVSIFNVGCAVGGIFLCKIADVYG RRIGLMFSMVVYVVGIIIQIASTTKWYQYFIGRLIAGLAVGTVSVISPLFISEV APKQLRGTLVCCFQLCITLGIFLGYCTTYGTKTYTDSRQWRIPLGICFAWAL FLVAGMLNMPESPRYLVEKSRIDDARKSIARSNKVSEEDPAVYTEVQLIQA GIDREALAGSATWMELVTGKPKIFRRVIMGVMLQSLQQLTGDNYFFYYGT TIFKAVGLQDSFQTSIILGIVNFASTFVGIYAIERMGRRLCLLTGSACMFVCFI IYSLIGTQHLYKNGFSNEPSNTYKPSGNAMIFITCLYIFFFASTWAGGVYCIV SESYPLRIRSKAMSVATAANWMWGFLISFFTPFITSAIHFYYGFVFTGCLAFS FFYVYFFVVETKGLSLEEVDILYASGTLPWKSSGWVP

4	Amino acid sequence of *H0 Metschnikowia species* Gxf2p/Gal2p	MSAEQEQQVSGTSATIDGLASLKQEKTAEEEDAFKPKPATAYFFISFLCGLV AFGGYVFGFDTGTISGFVNMDDYLMRFGQQHADGTYYLSNVRTGLIVSIFN IGCAVGGLALSKVGDIWGRRIGIMVAMIIYMVGIIIQIASQDKWYQYFIGRLI TGLGVGTTSVLSPLFISESAPKHLRGTLVCCFQLMVTLGIFLGYCTTYGTKN YTDSRQWRIPLGLCFAWALLLISGMVFMPESPRFLIERQRFDEAKASVAKS NQVSTEDPAVYTEVELIQAGIDREALAGSAGWKELITGKPKMLQRVILGM MLQSIQQLTGNNYFFYYGTTIFKAVGMSDSFQTSIVLGIVNFASTFVGIWAI ERMGRRSCLLVGSACMSVCFLIYSILGSVNLYIDGYENTPSNTRKPTGNAMI FITCLFIFFFASTWAGGVYSIVSETYPLRIRSKGMAVATAANWMWGFLISFF TPFITSAIHFYYGFVFTGCLIFSFFYVFFFVRETKGLSLEEVDELYATDLPPW KTAGWTPPSAEDMAHTTGFAEAAKPTNKHV
5	Amino acid sequence of *H0 Metschnikowia species* ΔGxs1p/ΔHgt12p (variant of Gxs1p/Hgt12p with shorter N-terminus)	MGIFVGVFAALGGVLFGYDTGTISGVMAMPWVKEHFPKDRVAFSASESSLI VSILSAGTFFGAILAPLLTDTLGRRWCIIISSLVVFNLGAALQTAATDIPLLIV GRVIAGLGVGLISSTIPLYQSEALPKWIRGAVVSCYQWAITIGIFLAAVINQG THKINSPASYRIPLGIQMAWGLILGVGMFFLPETPRFYISKGQNAKAAVSLA RLRKLPQDHPELLEELEDIQAAYEFETVHGKSSWSQVFTNKNKQLKKLATG VCLQAFQQLTGVNFIFYFGTTFFNSVGLDGFTTSLATNIVNVGSTIPGILGVE IFGRRKVLLTGAAGMCLSQFIVAIVGVATDSKAANQVLIAFCCIFIAFFAAT WGPTAWVVCGEIFPLRTRAKSIAMCAASNWLLNWAIAYATPYLVDSDKG NLGTNVFFIWGSCNFFCLVFAYFMIYETKGLSLEQVDELYEKVASARKSPG FVPSEHAFREHADVETAMPDNFNLKAEAISVEDASV
6	NOT USED	
7	Amino acid sequence of *H0 Metschnikowia species* Gxs1p/Hgt12	MGLESNKLIRKYINVGEKRAGSSGMGIFVGVFAALGGVLFGYDTGTISGVM AMPWVKEHFPKDRVAFSASESSLIVSILSAGTFFGAILAPLLTDTLGRRWCII ISSLVVFNLGAALQTAATDIPLLIVGRVIAGLGVGLISSTIPLYQSEALPKWIR GAVVSCYQWAITIGIFLAAVINQGTHKINSPASYRIPLGIQMAWGLILGVGM FFLPETPRFYISKGQNAKAAVSLARLRKLPQDHPELLEELEDIQAAYEFETV HGKSSWSQVFTNKNKQLKKLATGVCLQAFQQLTGVNFIFYFGTTFFNSVG LDGFTTSLATNIVNVGSTIPGILGVEIFGRRKVLLTGAAGMCLSQFIVAIVGV ATDSKAANQVLIAFCCIFIAFFAATWGPTAWVVCGEIFPLRTRAKSIAMCAA SNWLLNWAIAYATPYLVDSDKGNLGTNVFFIWGSCNFFCLVFAYFMIYET KGLSLEQVDELYEKVASARKSPGFVPSEHAFREHADVETAMPDNFNLKAE AISVEDASV
8	Amino acid sequence of *H0 Metschnikowia species* Hxt5p	MSIFEGKDGKGVSSTESLSNDVRYDNMEKVDQDVLRHNFNFDKEFEELEIE AAQVNDKPSFVDRILSLEYKLHFENKNHMVWLLGAFAAAAGLLSGLDQSII SGASIGMNKALNLTEREASLVSSLMPLGAMAGSMIMTPLNEWFGRKSSLIIS CIWYTIGSALCAGARDHHMMYAGRFILGVGVGIEGGCVGIYISESVPANVR GSIVSMYQFNIALGEVLGYAVAAIFYTVHGGWRFMVGSSLVFSTILFAGLFF LPESPRWLVHKGRNGMAYDVWKRLRDINDESAKLEFLEMRQAAYQERER RSQESLFSSWGELFTIARNRRALTYSVIMITLGQLTGVNAVMYYMSTLMGA IGFNEKDSVFMSLVGGGSLLIGTIPAILWMDRFGRRVWGYNLVGFFVGLVL VGVGYRFNPVTQKAASEGVYLTGLIVYFLFFGSYSTLTWVIPSESFDLRTRS LGMTICSTFLYLWSFTVTYNFTKMSAAFTYTGLTLGFYGGIAFLGLIYQVCF MPETKDKTLEEIDDIFNRSAFSIARENISNLKKGIW
9	Amino acid sequence of *H0 Metschnikowia species* Xyt1p with S75L mutation.	MGYEEKLVAPALKFKNFLDKTPNIHNVYVIAAISCTSGMMFGFDISSMSVF VDQQPYLKMFDNPSSVIQGFITALMSLGSFFGSLTSTFISEPFGRRASLFICGI LWVIGAAVQSSSQNRAQLICGRIIAGWGIGFGSSVAPVYGSEMAPRKIRGTI GGIFQFSVTVGIFIMFLIGYGCSFIQGKASFRIPWGVQMVPGLILLIGLFFIPES PRWLAKQGYWEDAEIIVANVQAKGNRNDANVQIEMSEIKDQLMLDEHLK EFTYADLFTKKYRQRTITAIFAQIWQQLTGMNVMMYYIVYIFQMAGYSGN TNLVPSLIQYIINMAVTVPALFCLDLLGRRTILLAGAAFMMAWQFGVAGIL ATYSEPAYISDTVRITIPDDHKSAAKGVIACCYLFVCSFAFSWGVGIWVYCS EVWGDSQSRQRGAALATSANWIFNFAIAMFTPSSFKNITWKTYIIYATFCAC

		MFIHVFFFFPETKGKRLEEIGQLWDEGVPAWRSAKWQPTVPLASDAELAH KMDVAHAEHADLLATHSPSSDEKTGTV
10	Amino acid sequence of *H0 Metschnikowia species* Hxt2.6p	MSSTTDTLEKRDTEPFTSDAPVTVHDYIAEERPWWKVPHLRVLTWSVFVIT LTSTNNGYDGSMLNGLQSLDIWQEDLGHPAGQKLGALANGVLFGNLAAV PFASYFCDRFGRRPVICFGQILTIVGAVLQGLSNSYGFFLGSRIVLGFGAMIA TIPSPTLISEIAYPTHRETSTFAYNVCWYLGAIIASWVTYGTRDLQSKACWSI PSYLQAALPFFQVCMIWFVPESPRFLVAKGKIDQARAVLSKYHTGDSTDPR DVALVDFELHEIESALEQEKLNTRSSYFDFFKKRNFRKRGFLCVMVGVAM QLSGNGLVSYYLSKVLDSIGITETKRQLEINGCLMIYNFVICVSLMSVCRMF KRRVLFLTCFSGMTVCYTIWTILSALNEQRHFEDKGLANGVLAMIFFYYFF YNVGINGLPFLYITEILPYSHRAKGLNLFQFSQFLTQIYNGYVNPIAMDAISW KYYIVYCCILFVELVIVFFTFPETSGYTLEEVAQVFGDEAPGLHNRQLDVAK ESLEHVEHV
11	Amino acid sequence of *H0 Metschnikowia species* Qup2p	MGFRNLKRRLSNVGDSMSVHSVKEEEDFSRVEIPDEIYNYKIVLVALTAAS AAIIIGYDAGFIGGTVSLTAFKSEFGLDKMSATAASAIEANVVSVFQAGAYF GCLFFYPIGEIWGRKIGLLLSGFLLTFGAAISLISNSSRGLGAIYAGRVLTGLG IGGCSSLAPIYVSEIAPAAIRGKLVGCWEVSWQVGGIVGYWINYGVLQTLPI SSQQWIIPFAVQLIPSGLFWGLCLLIPESPRFLVSKGKIDKARKNLAYLRGLS EDHPYSVFELENISKAIEENFEQTGRGFFDPLKALFFSKKMLYRLLLSTSMF MMQNGYGINAVTYYSPTIFKSLGVQGSNAGLLSTGIFGLLKGAASVFWVFF LVDTFGRRFCLCYLSLPCSICMWYIGAYIKIANPSAKLAAGDTATTPAGTAA KAMLYIWTIFYGITWNGTTWVICAEIFPQSVRTAAQAVNASSNWFWAFMI GHFTGQALENIGYGYYFLFAACSAIFPVVVWFVYPETKGVPLEAVEYLFEV RPWKAHSYALEKYQIEYNEGEFHQHKPEVLLQGSENSD
12	Amino acid sequence of *H0 Metschnikowia species* Aps1p/Hgt19p	MGYEEKLVAPALKFKNFLDKTPNIHNVYVIAAISCTSGMMFGFDISSMSVF VDQQPYLKMFDNPSSVIQGFITASMSLGSFFGSLTSTFISEPFGRRASLFICGI LWVIGAAVQSSSQNRAQLICGRIIAGWGIGFGSSVAPVYGSEMAPRKIRGTI GGIFQFSVTVGIFIMFLIGYGCSFIQGKASFRIPWGVQMVPGLILLIGLFFIPES PRWLAKQGYWEDAEIIVANVQAKGNRNDANVQIEMSEIKDQLMLDEHLK EFTYADLFTKKYRQRTITAIFAQIWQQLTGMNVMMYYIVYIFQMAGYSGN TNLVPSLIQYIINMAVTVPALFCLDLLGRRTILLAGAAFMMAWQFGVAGIL ATYSEPAYISDTVRITIPDDHKSAAKGVIACCYLFVCSFAFSWGVGIWVYCS EVWGDSQSRQRGAALATSANWIFNFAIAMFTPSSFKNITWKTYIIYATFCAC MFIHVFFFFPETKGKRLEEIGQLWDEGVPAWRSAKWQPTVPLASDAELAH KMDVAHAEHADLLATHSPSSDEKTGTV
13	Nucleic acid sequence of *H0 Metschnikowia species XYT1*	ATGGGTTACGAGGAAAAGCTTGTAGCGCCCGCGTTGAAATTCAAAAAC TTTCTTGACAAAACCCCCAATATTCACAATGTCTATGTCATTGCCGCCAT CTCCTGTACATCAGGTATGATGTTTGGATTTGATATCTCGTCGATGTCTG TCTTTGTCGACCAGCAGCCATACTTGAAGATGTTTGACAACCCTAGTTC CGTGATTCAAGGTTTCATTACCGCGCTGATGAGTTTGGGCTCGTTTTTCG GCTCGCTCACATCCACGTTCATCTCTGAGCCTTTTGGTCGTCGTGCATCG TTGTTCATTTGTGGTATTCTTTGGGTAATTGGAGCAGCGGTTCAAAGTTC GTCGCAGAACAGGGCCCAATTGATTTGTGGGCGTATCATTGCAGGATGG GGCATTGGCTTTGGGTCATCGGTGGCTCCTGTTTACGGGTCCGAGATGG CTCCGAGAAAGATCAGAGGCACGATTGGTGGAATCTTCCAGTTCTCCGT CACCGTGGGTATCTTTATCATGTTCTTGATTGGGTACGGATGCTCTTTCA TTCAAGGAAAGGCCTCTTTCCGGATCCCCTGGGGTGTGCAAATGGTTCC CGGCCTTATCCTCTTGATTGGACTTTTCTTTATTCCTGAATCTCCCCGTTG GTTGGCCAAACAGGGCTACTGGGAAGACGCCGAAATCATTGTGGCCAA TGTGCAGGCCAAGGGTAACCGTAACGACGCCAACGTGCAGATTGAAAT GTCGGAGATTAAGGATCAATTGATGCTTGACGAGCACTTGAAGGAGTTT ACGTACGCTGACCTTTTCACGAAGAAGTACCGCCAGCGCACGATCACGG CGATCTTTGCCCAGATCTGGCAACAGTTGACCGGTATGAATGTGATGAT GTACTACATTGTGTACATTTTCCAGATGGCAGGCTACAGCGGCAACACG AACTTGGTGCCCAGTTTGATCCAGTACATCATCAACATGGCGGTCACGG

		TGCCGGCGCTTTTCTGCTTGGATCTCTTGGGCCGTCGTACCATTTTGCTC GCGGGTGCCGCGTTCATGATGGCGTGGCAATTCGGCGTGGCGGGCATTT TGGCCACTTACTCAGAACCGGCATATATCTCTGACACTGTGCGTATCAC GATCCCCGACGACCACAAGTCTGCTGCAAAAGGTGTGATTGCATGCTGC TATTTGTTTGTGTGCTCGTTTGCATTCTCGTGGGGTGTCGGTATTTGGGT GTACTGTTCCGAGGTTTGGGGTGACTCCCAGTCGAGACAAAGAGGCGCC GCTCTTGCGACGTCGGCCAACTGGATCTTCAACTTCGCCATTGCCATGTT CACGCCGTCCTCATTCAAGAATATCACGTGGAAGACGTATATCATCTAC GCCACGTTCTGTGCGTGCATGTTCATACACGTGTTTTTCTTTTTCCCAGA AACAAAGGGCAAGCGTTTGGAGGAGATAGGCCAGCTTTGGGACGAAGG AGTCCCAGCATGGAGGTCAGCCAAGTGGCAGCCAACAGTGCCGCTCGC GTCCGACGCAGAGCTTGCACACAAGATGGATGTTGCGCACGCGGAGCA CGCGGACTTATTGGCCACGCACTCGCCATCTTCAGACGAGAAGACGGGC ACGGTCTAA
14	Nucleic acid sequence of *H0 Metschnikowia species GXF1*	ATGTCTCAAGACGAACTTCATACAAAGTCTGGTGTTGAAACACCAATCA ACGATTCGCTTCTCGAGGAGAAGCACGATGTCACCCCACTCGCGGCATT GCCCGAGAAGTCCTTCAAGGACTACATTTCCATTTCCATTTTCTGTTTGT TTGTGGCATTTGGTGGTTTTGTTTTCGGTTTCGACACCGGTACGATTTCC GGTTTCGTCAACATGTCCGACTTCAAGACCAGATTTGGTGAGATGAATG CCCAGGGCGAATACTACTTGTCCAATGTTAGAACTGGTTTGATGGTTTC TATTTTCAACGTCGGTTGCGCCGTTGGTGGTATCTTCCTTTGTAAGATTG CCGATGTTTATGGCAGAAGAATTGGTCTTATGTTTTCCATGGTGGTTTAT GTCGTTGGTATCATTATTCAGATTGCCTCCACCACCAAATGGTACCAAT ACTTCATTGGCCGTCTTATTGCTGGCTTGGCTGTGGGTACTGTTTCCGTC ATCTCGCCACTTTTCATTTCCGAGGTTGCTCCTAAACAGCTCAGAGGTAC GCTTGTGTGCTGCTTCCAGTTGTGTATCACCTTGGGTATCTTTTTGGGTT ACTGCACGACCTACGGTACAAAGACTTACACTGACTCCAGACAGTGGA GAATCCCATTGGGTATCTGTTTCGCGTGGGCTTTGTTTTTGGTGGCCGGT ATGTTGAACATGCCCGAGTCTCCTAGATACTTGGTTGAGAAATCGAGAA TCGACGATGCCAGAAAGTCCATTGCCAGATCCAACAAGGTTTCCGAGG AAGACCCCGCCGTGTACACCGAGGTGCAGCTTATCCAGGCTGGTATTGA CAGAGAGGCCCTTGCCGGCAGCGCCACATGGATGGAGCTTGTGACTGG TAAGCCCAAAATCTTCAGAAGAGTCATCATGGGTGTCATGCTTCAGTCC TTGCAACAATTGACTGGTGACAACTACTTTTTCTACTACGGAACCACGA TTTTCAAGGCTGTTGGCTTGCAGGACTCTTTCCAGACGTCGATTATCTTG GGTATTGTCAACTTTGCCTCGACTTTTGTCGGTATTTACGCCATTGAGAG AATGGGCAGAAGATTGTGTTTGTTGACCGGATCTGCGTGCATGTTTGTG TGTTTCATCATCTACTCGCTCATTGGTACGCAGCACTTGTACAAGAACG GCTTCTCTAACGAACCTTCCAACACATACAAGCCTTCCGGTAACGCCAT GATCTTCATCACGTGTCTTTACATTTTCTTCTTTGCCTCGACCTGGGCCG GTGGTGTTTACTGTATCGTGTCCGAGTCTTACCCATTGAGAATCAGATCC AAGGCCATGTCTGTCGCCACCGCCGCCAACTGGATGTGGGGTTTCTTGA TCTCGTTCTTCACGCCTTTCATCACCTCCGCCATCCACTTTTACTACGGTT TTGTTTTCACTGGCTGCTTGGCGTTCTCCTTCTTCTACGTCTACTTCTTTG TCGTGGAGACCAAGGGTCTTTCCTTGGAGGAGGTTGACATTTTGTACGC TTCCGGTACGCTTCCATGGAAGTCCTCTGGCTGGGTGCCTCCTACCGCG GACGAAATGGCCCACAACGCCTTCGACAACAAGCCAACTGACGAACAA GTCTAA
15	Nucleic acid sequence of *H0 Metschnikowia species ΔGXF1* (variant of *GXF1* with shorter N-terminus)	ATGTCCGACTTCAAGACCAGATTTGGTGAGATGAATGCCCAGGGCGAAT ACTACTTGTCCAATGTTAGAACTGGTTTGATGGTTTCTATTTTCAACGTC GGTTGCGCCGTTGGTGGTATCTTCCTTTGTAAGATTGCCGATGTTTATGG CAGAAGAATTGGTCTTATGTTTTCCATGGTGGTTTATGTCGTTGGTATCA TTATTCAGATTGCCTCCACCACCAAATGGTACCAATACTTCATTGGCCGT CTTATTGCTGGCTTGGCTGTGGGTACTGTTTCCGTCATCTCGCCACTTTT CATTTCCGAGGTTGCTCCTAAACAGCTCAGAGGTACGCTTGTGTGCTGC TTCCAGTTGTGTATCACCTTGGGTATCTTTTTGGGTTACTGCACGACCTA CGGTACAAAGACTTACACTGACTCCAGACAGTGGAGAATCCCATTGGGT ATCTGTTTCGCGTGGGCTTTGTTTTTGGTGGCCGGTATGTTGAACATGCC

		CGAGTCTCCTAGATACTTGGTTGAGAAATCGAGAATCGACGATGCCAGA AAGTCCATTGCCAGATCCAACAAGGTTTCCGAGGAAGACCCCGCCGTGT ACACCGAGGTGCAGCTTATCCAGGCTGGTATTGACAGAGAGGCCCTTGC CGGCAGCGCCACATGGATGGAGCTTGTGACTGGTAAGCCCAAAATCTTC AGAAGAGTCATCATGGGTGTCATGCTTCAGTCCTTGCAACAATTGACTG GTGACAACTACTTTTTCTACTACGGAACCACGATTTTCAAGGCTGTTGG CTTGCAGGACTCTTTCCAGACGTCGATTATCTTGGGTATTGTCAACTTTG CCTCGACTTTTGTCGGTATTTACGCCATTGAGAGAATGGGCAGAAGATT GTGTTTGTTGACCGGATCTGCGTGCATGTTTGTGTGTTTCATCATCTACT CGCTCATTGGTACGCAGCACTTGTACAAGAACGGCTTCTCTAACGAACC TTCCAACACATACAAGCCTTCCGGTAACGCCATGATCTTCATCACGTGT CTTTACATTTTCTTCTTTGCCTCGACCTGGGCCGGTGGTGTTTACTGTAT CGTGTCCGAGTCTTACCCATTGAGAATCAGATCCAAGGCCATGTCTGTC GCCACCGCCGCCAACTGGATGTGGGGTTTCTTGATCTCGTTCTTCACGCC TTTCATCACCTCCGCCATCCACTTTTACTACGGTTTTGTTTTCACTGGCTG CTTGGCGTTCTCCTTCTTCTACGTCTACTTCTTTGTCGTGGAGACCAAGG GTCTTTCCTTGGAGGAGGTTGACATTTTGTACGCTTCCGGTACGCTTCCA TGGAAGTCCTCTGGCTGGGTGCCTCCTACCGCGGACGAAATGGCCCACA ACGCCTTCGACAACAAGCCAACTGACGAACAAGTCTAA
16	Nucleic acid sequence of *H0 Metschnikowia species GXF2/GAL2*	ATGAGTGCCGAACAGGAACAACAAGTATCGGGCACATCTGCCACGATA GATGGGCTGGCGTCCTTGAAGCAAGAAAAAACCGCCGAGGAGGAAGAC GCCTTCAAGCCTAAGCCCGCCACGGCGTACTTTTTCATTTCGTTCCTCTG TGGCTTGGTCGCCTTTGGCGGCTACGTTTTCGGTTTCGATACCGGTACGA TTTCCGGGTTTGTTAACATGGACGACTATTTGATGAGATTCGGCCAGCA GCACGCTGATGGCACGTATTACCTTTCCAACGTGAGAACCGGTTTGATC GTGTCGATCTTCAACATTGGCTGTGCCGTTGGTGGTCTTGCGCTTTCGAA AGTCGGTGACATTTGGGGCAGAAGAATTGGTATTATGGTTGCTATGATC ATCTACATGGTGGGAATCATCATCCAGATCGCTTCACAGGATAAATGGT ACCAGTACTTCATTGGCCGTTTGATCACCGGATTGGGTGTCGGCACCAC GTCCGTGCTTAGTCCTCTTTTCATCTCCGAGTCGGCTCCGAAGCATTTGA GAGGCACCCTTGTGTGTTGTTTCCAGCTCATGGTCACCTTGGGTATCTTT TTGGGCTACTGCACGACCTACGGTACCAAGAACTACACTGACTCGCGCC AGTGGCGGATTCCCTTGGGTCTTTGCTTCGCATGGGCTCTTTTGTTGATC TCGGGAATGGTTTTCATGCCTGAATCCCCACGTTTCTTGATTGAGCGCCA GAGATTCGACGAGGCCAAGGCTTCCGTGGCCAAATCGAACCAGGTTTC GACCGAGGACCCCGCCGTGTACACTGAAGTCGAGTTGATCCAGGCCGG TATTGACCGTGAGGCATTGGCCGGATCCGCTGGCTGGAAAGAGCTTATC ACGGGTAAGCCCAAGATGTTGCAGCGTGTGATTTTGGGAATGATGCTCC AGTCGATCCAGCAGCTTACCGGTAACAACTACTTTTTCTACTATGGTAC CACGATCTTCAAGGCCGTGGGCATGTCGGACTCGTTCCAGACCTCGATT GTTTTGGGTATTGTCAACTTCGCCTCCACTTTTGTCGGAATCTGGGCCAT CGAACGCATGGGCCGCAGATCTTGTTTGCTTGTTGGTTCCGCGTGCATG AGTGTGTGTTTCTTGATCTACTCCATCTTGGGTTCCGTCAACCTTTACAT CGACGGCTACGAGAACACGCCTTCCAACACGCGTAAGCCTACCGGTAA CGCCATGATTTTCATCACGTGTTTGTTCATCTTCTTCTTCGCCTCCACCTG GGCCGGTGGTGTGTACAGTATTGTGTCTGAAACATACCCATTGAGAATC CGCTCTAAAGGTATGGCCGTGGCCACCGCTGCCAACTGGATGTGGGGTT TCTTGATTTCGTTCTTCACGCCTTTCATCACCTCGGCCATCCACTTCTACT ACGGGTTTGTGTTCACAGGGTGTCTTATTTTCTCCTTCTTCTACGTGTTCT TCTTTGTTAGGGAAACCAAGGGTCTCTCGTTGGAAGAGGTGGATGAGTT ATATGCCACTGACCTCCCACCATGGAAGACCGCGGGCTGGACGCCTCCT TCTGCTGAGGATATGGCCCACACCACCGGGTTTGCCGAGGCCGCAAAGC CTACGAACAAACACGTTTAA
17	Nucleic acid sequence of *H0 Metschnikowia species ΔGXS1*	ATGGGCATTTTCGTTGGCGTTTTCGCCGCGCTTGGCGGTGTTCTCTTTGG CTACGATACCGGTACCATCTCTGGTGTGATGGCCATGCCTTGGGTCAAG GAACATTTCCCAAAAGACCGTGTTGCATTTAGTGCTTCCGAGTCGTCGT TGATTGTGTCTATTTTATCTGCAGGAACTTTCTTTGGAGCCATTCTTGCT

	/ΔHGT12 (variant of *GXS1* /*HGT12* with shorter N-terminus)	CCGCTCTTGACCGATACATTGGGTAGACGCTGGTGTATTATCATCTCTTC GCTCGTTGTGTTCAATTTGGGTGCTGCTTTGCAGACGGCTGCCACGGAT ATCCCGCTCTTGATTGTTGGTCGTGTCATTGCCGGTTTAGGGGTTGGTTT GATTTCGCTGACGATTCCATTGTACCAGTCCGAAGCGCTTCCCAAATGG ATTAGAGGTGCTGTTGTCTCGTGCTACCAATGGGCCATTACTATTGGTAT CTTTTTGGCTGCCGTGATCAACCAGGGCACTCACAAGATCAACAGCCCT GCGTCGTACAGAATTCCATTGGGTATTCAGATGGCATGGGGTCTTATCT TGGGTGTCGGCATGTTCTTCTTGCCCGAGACGCCTCGTTTCTACATTTCC AAGGGCCAGAATGCGAAGGCTGCTGTTTCATTGGCGCGTTTGAGAAAG CTTCCGCAAGATCACCCGGAGTTGTTGGAGGAATTGGAAGATATCCAGG CGGCATACGAGTTTGAGACTGTCCATGGCAAGTCTTCATGGCTGCAGGT TTTCACCAACAAGAACAAACAATTGAAGAAGTTGGCCACGGGCGTGTG CTTGCAGGCGTTCCAACAATTGACTGGTGTGAACTTCATTTTCTACTTTG GCACGACTTTCTTCAACAGTGTTGGGCTTGACGGATTCACCACCTCCTTG GCCACCAACATTGTCAATGTTGGCTCGACGATCCCTGGTATTTTGGGTG TTGAGATTTTCGGCAGAAGAAAAGTGTTGTTGACCGGCGCTGCTGGTAT GTGTCTTTCGCAATTCATTGTTGCCATTGTTGGTGTAGCCACCGACTCCA AGGCTGCGAACCAAGTTCTTATTGCCTTCTGCTGCATTTTCATTGCGTTC TTTGCAGCCACCTGGGGCCCCACCGCATGGGTTGTTTGTGGCGAGATTT TCCCCTTGAGAACCAGAGCCAAGTCGATTGCCATGTGCGCTGCTTCGAA CTGGTTGTTGAACTGGGCAATTGCATACGCCACGCCATACTTGGTTGAC TCCGATAAGGGTAACTTGGGCACCAATGTGTTTTTCATTTGGGGAAGCT GTAACTTCTTCTGCCTTGTGTTTGCCTACTTCATGATTTACGAGACCAAG GGTCTTTCCTTGGAGCAGGTTGATGAGCTTTACGAGAAGGTTGCCAGCG CCAGAAAGTCGCCTGGCTTCGTGCCAAGCGAGCACGCTTTCAGAGAGC ACGCCGATGTGGAGACCGCCATGCCAGACAACTTCAACTTGAAGGCGG AGGCGATTTCTGTCGAGGATGCCTCTGTTTAA
18	NOT USED	
19	Nucleic acid sequence of *H0 Metschnikowia species GXS1/HGT12*	ATGAGCATCTTTGAAGGCAAAGACGGGAAGGGGGTATCCTCCACCGAG TCGCTTTCCAATGACGTCAGATATGACAACATGGAGAAAGTTGATCAGG ATGTTCTTAGACACAACTTCAACTTTGACAAAGAATTCGAGGAGCTCGA AATCGAGGCGGCGCAAGTCAACGACAAACCTTCTTTTGTCGACAGGATT TTATCCCTCGAATACAAGCTTCATTTCGAAAACAAGAACCACATGGTGT GGCTCTTGGGCGCTTTCGCAGCCGCCGCAGGCTTATTGTCTGGCTTGGA TCAGTCCATTATTTCTGGTGCATCCATTGGAATGAACAAAGCATTGAAC TTGACTGAACGTGAAGCCTCATTGGTGTCTTCGCTTATGCCTTTAGGCGC CATGGCAGGCTCCATGATTATGACACCTCTTAATGAGTGGTTCGGAAGA AAATCATCGTTGATTATTTCTTGTATTTGGTATACCATCGGATCCGCTTT GTGCGCTGGCGCCAGAGATCACCACATGATGTACGCTGGCAGATTTATT CTTGGTGTCGGTGTGGGTATAGAAGGTGGGTGTGTGGGCATTTACATTT CCGAGTCTGTCCCAGCCAATGTGCGTGGTAGTATCGTGTCGATGTACCA GTTCAATATTGCTTTGGGTGAAGTTCTAGGGTATGCTGTTGCTGCCATTT TCTACACTGTTCATGGTGGATGGAGGTTCATGGTGGGGTCTTCTTTAGTA TTCTCTACTATATTGTTTGCCGGATTGTTTTTCTTGCCCGAGTCACCTCGT TGGTTGGTGCACAAAGGCAGAAACGGAATGGCATACGATGTGTGGAAG AGATTGAGAGACATAAACGATGAAAGCGCAAAGTTGGAATTTTTGGAG ATGAGACAGGCTGCTTATCAAGAGAGAGAAAGACGCTCGCAAGAGTCT TTGTTCTCCAGCTGGGGCGAATTATTCACCATCGCTAGAAACAGAAGAG CACTTACTTACTCTGTCATAATGATCACTTTGGGTCAATTGACTGGTGTC AATGCCGTCATGTACTACATGTCGACTTTGATGGGTGCAATTGGTTTCA ACGAGAAAGACTCTGTGTTCATGTCCCTTGTGGGAGGCGGTTCTTTGCT TATAGGTACCATTCCTGCCATTTTGTGGATGGACCGTTTCGGCAGAAGA GTTTGGGGTTATAATCTTGTTGGTTTCTTCGTTGGTTTGGTGCTCGTTGG TGTTGGCTACCGTTTCAATCCCGTCACTCAAAAGGCGGCTTCAGAAGGT GTGTACTTGACGGGTCTCATTGTCTATTTCTTGTTCTTTGGTTCCTACTCG ACCTTAACTTGGGTCATTCCATCCGAGTCTTTTGATTTGAGAACAAGATC

		TTTGGGTATGACAATCTGTTCCACTTTCCTTTACTTGTGGTCTTTCACCGT CACCTACAACTTCACCAAGATGTCCGCCGCCTTCACATACACTGGGTTG ACACTTGGTTTCTACGGTGGCATTGCGTTCCTTGGTTTGATTTACCAGGT CTGCTTCATGCCCGAGACGAAGGACAAGACTTTGGAAGAAATTGACGA TATCTTCAATCGTTCTGCGTTCTCTATCGCGCGCGAGAACATCTCCAACT TGAAGAAGGGTATTTGGTAA
20	Nucleic acid sequence of *H0 Metschnikowia species HXT5*	ATGAGCATCTTTGAAGGCAAAGACGGGAAGGGGGTATCCTCCACCGAG TCGCTTTCCAATGACGTCAGATATGACAACATGGAGAAAGTTGATCAGG ATGTTCTTAGACACAACTTCAACTTTGACAAAGAATTCGAGGAGCTCGA AATCGAGGCGGCGCAAGTCAACGACAAACCTTCTTTTGTCGACAGGATT TTATCCCTCGAATACAAGCTTCATTTCGAAAACAAGAACCACATGGTGT GGCTCTTGGGCGCTTTCGCAGCCGCCGCAGGCTTATTGTCTGGCTTGGA TCAGTCCATTATTTCTGGTGCATCCATTGGAATGAACAAAGCATTGAAC TTGACTGAACGTGAAGCCTCATTGGTGTCTTCGCTTATGCCTTTAGGCGC CATGGCAGGCTCCATGATTATGACACCTCTTAATGAGTGGTTCGGAAGA AAATCATCGTTGATTATTTCTTGTATTTGGTATACCATCGGATCCGCTTT GTGCGCTGGCGCCAGAGATCACCACATGATGTACGCTGGCAGATTTATT CTTGGTGTCGGTGTGGGTATAGAAGGTGGGTGTGTGGGCATTTACATTT CCGAGTCTGTCCCAGCCAATGTGCGTGGTAGTATCGTGTCGATGTACCA GTTCAATATTGCTTTGGGTGAAGTTCTAGGGTATGCTGTTGCTGCCATTT TCTACACTGTTCATGGTGGATGGAGGTTCATGGTGGGGTCTTCTTTAGTA TTCTCTACTATATTGTTTGCCGGATTGTTTTTCTTGCCCGAGTCACCTCGT TGGTTGGTGCACAAAGGCAGAAACGGAATGGCATACGATGTGTGGAAG AGATTGAGAGACATAAACGATGAAAGCGCAAAGTTGGAATTTTTGGAG ATGAGACAGGCTGCTTATCAAGAGAGAGAAAGACGCTCGCAAGAGTCT TTGTTCTCCAGCTGGGGCGAATTATTCACCATCGCTAGAAACAGAAGAG CACTTACTTACTCTGTCATAATGATCACTTTGGGTCAATTGACTGGTGTC AATGCCGTCATGTACTACATGTCGACTTTGATGGGTGCAATTGGTTTCA ACGAGAAAGACTCTGTGTTCATGTCCCTTGTGGGAGGCGGTTCTTTGCT TATAGGTACCATTCCTGCCATTTTGTGGATGGACCGTTTCGGCAGAAGA GTTTGGGGTTATAATCTTGTTGGTTTCTTCGTTGGTTTGGTGCTCGTTGG TGTTGGCTACCGTTTCAATCCCGTCACTCAAAAGGCGGCTTCAGAAGGT GTGTACTTGACGGGTCTCATTGTCTATTTCTTGTTCTTTGGTTCCTACTCG ACCTTAACTTGGGTCATTCCATCCGAGTCTTTTGATTTGAGAACAAGATC TTTGGGTATGACAATCTGTTCCACTTTCCTTTACTTGTGGTCTTTCACCGT CACCTACAACTTCACCAAGATGTCCGCCGCCTTCACATACACTGGGTTG ACACTTGGTTTCTACGGTGGCATTGCGTTCCTTGGTTTGATTTACCAGGT CTGCTTCATGCCCGAGACGAAGGACAAGACTTTGGAAGAAATTGACGA TATCTTCAATCGTTCTGCGTTCTCTATCGCGCGCGAGAACATCTCCAACT TGAAGAAGGGTATTTGGTAA
21	Nucleic acid sequence of *H0 Metschnikowia species XYT1* codon optimized for expression in *S. cerevisiae*	ATGGGATACGAAGAGAAATTAGTGGCCCCCGCTTTGAAATTTAAGAACT TTTTGGATAAGACCCCAAATATACATAACGTTTACGTAATTGCGGCGAT CTCGTGTACCTCAGGTATGATGTTCGGTTTCGATATATCGTCGATGTCCG TGTTCGTGGACCAACAGCCGTATTTAAAAATGTTTGATAACCCTAGCAG CGTGATACAAGGGTTTATAACTGCGTTGATGTCTTTGGGGAGCTTTTTCG GATCGCTAACGTCCACTTTTATTTCAGAACCTTTTGGTAGACGTGCCTCT TTGTTCATATGCGGGATCCTTTGGGTAATTGGGGCGGCAGTTCAAAGTT CTTCTCAGAACCGTGCGCAGCTTATTTGTGGCCGAATTATTGCAGGGTG GGGCATCGGATTCGGTTCTAGCGTTGCGCCGGTATACGGTTCAGAAATG GCCCCACGCAAAATTAGAGGAACAATCGGAGGTATTTTTCAATTTTCTG TCACGGTCGGAATATTCATAATGTTCCTGATTGGCTACGGCTGCTCATTT ATACAAGGCAAGGCCAGTTTTAGAATTCCGTGGGGAGTTCAAATGGTAC CAGGTCTCATTCTGTTGATCGGACTATTCTTCATTCCTGAATCCCCAAGA TGGTTAGCCAAACAAGGCTACTGGGAAGACGCTGAGATCATCGTAGCA AACGTTCAAGCTAAGGGTAACAGGAACGATGCTAATGTGCAAATTGAA ATGTCCGAGATAAAAGATCAGTTAATGCTTGACGAGCATTTAAAGGAGT TTACTTATGCCGATTTGTTTACCAAAAAATACCGGCAAAGGACGATAAC AGCTATATTTGCCCAAATATGGCAACAGCTGACAGGTATGAATGTCATG

		ATGTACTACATCGTATATATATTTCAAATGGCAGGTTATTCAGGTAATA CTAATTTAGTTCCTTCACTCATTCAGTATATTATAAATATGGCTGTTACG GTCCCCGCATTGTTCTGTCTTGATCTGCTTGGCAGGAGGACAATTTTATT AGCTGGCGCCGCTTTTATGATGGCCTGGCAATTTGGTGTTGCTGGCATTT TAGCTACTTATTCAGAGCCAGCCTATATTTCAGATACCGTGAGAATTAC AATTCCAGATGACCATAAAAGTGCCGCTAAGGGTGTCATCGCTTGCTGC TATTTGTTTGTTTGTTCCTTCGCCTTTTCCTGGGGTGTAGGTATCTGGGTT TATTGTTCAGAAGTGTGGGGTGATAGTCAATCCAGACAAAGAGGTGCTG CATTGGCAACTTCTGCTAATTGGATCTTCAATTTCGCAATTGCAATGTTT ACACCTTCTTCTTTCAAAAATATCACTTGGAAGACTTATATCATTTATGC TACATTTTGTGCTTGTATGTTCATTCATGTTTTTTTTTTTTCCCTGAAAC AAAGGGTAAGAGACTAGAAGAAATTGGACAGCTATGGGATGAAGGTGT CCCAGCATGGAGATCTGCAAAATGGCAACCCACTGTCCCACTAGCAAGT GACGCTGAATTAGCTCACAAAATGGATGTTGCACACGCTGAACACGCA GACTTATTGGCAACCCATTCTCCAAGTAGTGACGAAAAAACTGGTACCG TTTAA
22	Nucleic acid sequence of *H0 Metschnikowia species HXT2.6*	ATGCTGAGCACTACCGATACCCTCGAAAAAAGGGACACCGAGCCTTTC ACTTCAGATGCTCCTGTCACAGTCCATGACTATATCGCAGAGGAGCGTC CGTGGTGGAAAGTGCCGCATTTGCGTGTATTGACTTGGTCTGTTTTCGTG ATCACCCTCACCTCCACCAACAACGGGTATGATGGCCTGATGTTGAATG GATTGCAATCCTTGGACATTTGGCAGGAGGATTTGGGTCACCCTGCGGG CCAGAAATTGGGTGCCTTGGCCAACGGTGTTTTGTTTGGTAACCTTGCT GCTGTGCCTTTTGCTTCGTATTTCTGCGATCGTTTTGGTAGAAGGCCGGT CATTTGTTTCGGACAGATCTTGACAATTGTTGGTGCTGTATTACAAGGTT TGTCCAACAGCTATGGATTTTTTTTGGGTTCGAGAATTGTGTTGGGTTTT GGTGCTATGATAGCCACTATTCCGCTGCCAACATTGATTTCCGAAATCG CCTACCCTACGCATAGAGAAACTTCCACTTTCGCCTACAACGTGTGCTG GTATTTGGGAGCCATTATCGCCTCCTGGGTCACATACGGCACCAGAGAT TTACAGAGCAAGGCTTGCTGGTCAATTCCTTCTTATCTCCAGGCCGCCTT ACCTTTCTTTCAAGTGTGCATGATTTGGTTTGTGCCAGAGTCTCCCAGAT TCCTCGTTGCCAAGGGCAAGATCGACCAAGCAAGGGCTGTTTTGTCTAA ATACCATACAGGAGACTCGACTGACCCCAGAGACGTTGCGTTGGTTGAC TTTGAGCTCCATGAGATTGAGAGTGCATTGGAGCAGGAAAAATTGAAC ACTCGCTCGTCATACTTTGACTTTTTCAAGAAGAGAAACTTTAGAAAGA GAGGCTTCTTGTGTGTCATGGTCGGTGTTGCAATGCAGCTTTCTGGAAA CGGCTTAGTGTCCTATTACTTGTCGAAAGTGCTAGACTCGATTGGAATC ACTGAAACCAAGAGACAGCTCGAGATCAATGGCTGCTTGATGATCTATA ACTTTGTCATCTGCGTCTCGTTGATGAGTGTTTGCCGTATGTTCAAAAGA AGAGTATTATTTCTCACGTGTTTCTCAGGAATGACGGTTTGCTACACGAT ATGGACGATTTTGTCAGCGCTTAATGAACAGAGACACTTTGAGGATAAA GGCTTGGCCAATGGCGTGTTGGCAATGATCTTCTTCTACTATTTTTTCTA CAACGTTGGCATCAATGGATTGCCATTCCTATACATCACCGAGATCTTG CCTTACTCACACAGAGCAAAAGGCTTGAATTTATTCCAATTCTCGCAAT TTCTCACGCAAATCTACAATGGCTATGTGAACCCAATCGCCATGGACGC AATCAGCTGGAAGTATTACATTGTGTACTGCTGTATTCTCTTCGTGGAGT TGGTGATTGTGTTTTTCACGTTCCCAGAAACTTCGGGATACACTTTGGAG GAGGTCGCCCAGGTATTTGGTGATGAGGCTCCCGGGCTCCACAACAGAC AATTGGATGTTGCGAAAGAATCACTCGAGCATGTTGAGCATGTTTGA
23	Nucleic acid sequence of *H0 Metschnikowia species HXT2.6* codon optimized for expression in *S. cerevisiae*	ATGAGCCAGTCTAAAGAAAAGTCCAACGTTATTACCACCGTCTTGTCTG AAGAATTGCCAGTTAAGTACTCCGAAGAAATCTCCGATTACGTTTACCA TGATCAACATTGGTGGAAGTACAACCACTTCAGAAAATTGCATTGGTAC ATCTTCGTTCTGACTTTGACTTCTACCAACAATGGTTACGATGGCTCTAT GTTGAACGGTCTACAATCTTTGTCTACTTGGAAAGATGCTATGGGTAAT CCTGAAGGTTACATTTTGGGTGCTTTGGCTAATGGTACTATTTTCGGTGG TGTTTTGGCTGTTGCTTTTGCTTCTTGGGCTTGTGATAGATTTGGTAGAA AGTTGACTACCTGCTTCGGTTCTATCGTTACTGTTATTGGTGCTATATTG CAAGGTGCCTCTACTAATTACGCATTCTTTTTCGTTTCCCGTATGGTTAT

		TGGTTTTGGTTTCGGTCTAGCTTCTGTTGCTTCTCCAACTTTGATTGCTGA ATTGTCTTTCCCAACTTACAGACCAACTTGTACTGCCTTGTACAATGTTT TTTGGTACTTGGGTGCTGTTATTGCTGCATGGGTTACTTATGGTACTAGA ACTATCGTTTCTGCCTACTCTTGGAGAATTCCATCTTACTTGCAAGGTTT GTTGCCATTGGTTCAAGTTTGTTTGGTTTGGTGGGTTCCAGAATCTCCAA GATTCTTGGTTTCTAAGGGTAAGATTGAAAAGGCCAGGGAATTCTTGAT TAAGTTCCATACTGGTAACGACACCCAAGAACAAGCTACTAGATTGGTC GAATTTGAGTTGAAAGAAATTGAAGCCGCCTTGGAGATGGAAAAGATT AACTCTAATTCTAAGTACACCGACTTCATCACCATCAAGACTTTCAGAA AGAGAATCTTCTTGGTTGCTTTCACTGCTTGTATGACTCAATTGTCTGGT AACGGTTTGGTGTCTTACTACTTGTCCAAGGTTTTGATCTCCATTGGTAT TACCGGTGAGAAAGAACAATTGCAAATCAACGGTTGCCTGATGATCTAC AACTTGGTTTTGTCTTTAGCTGTTGCCTTCACCTGTTACTTGTTTAGAAG AAAGGCCCTGTTCATCTTCTCTTGCTCATTCATGTTGTTGTCCTACGTTA TTTGGACCATTCTGTCCGCTATCAATCAACAGAGAAACTTCGAACAAAA AGGTCTAGGTCAAGGTGTCTTGGCTATGATTTTTATCTACTACTTGGCCT ACAACATCGGTTTGAATGGTTTGCCATACTTGTACGTTACCGAAATCTT GCCATATACTCATAGAGCTAAGGGCATCAACTTGTATTCCTTGGTTATT AACATCACCCTGATCTATAACGGTTTCGTTAACGCTATTGCTATGGATG CTATTTCCTGGAAGTACTACATCGTTTACTGCTGCATTATTGCCGTTGAA TTGGTTGTTGTTATCTTCACCTACGTTGAAACTTTCGGTTACACCTTGGA AGAAGTTGCTAGAGTTTTTGAAGGTACTGATTCTTTGGCCATGGACATT AACTTGAACGGTACAGTTTCCAACGAAAAGATCGATATCGTTCACTCTG AAAGAGGTTCCTCTGCTTAA
24	Nucleic acid sequence of *H0 Metschnikowia species QUP2*	ATGGGCTTTCGCAACTTAAAGCGCAGGCTCTCAAATGTTGGCGACTCCA TGTCAGTGCACTCTGTGAAAGAGGAGGAAGACTTCTCCCGCGTGGAAAT CCCGGATGAAATCTACAACTATAAGATCGTCCTTGTGGCTTTAACAGCG GCGTCGGCTGCCATCATCATCGGCTACGATGCAGGCTTCATTGGTGGCA CGGTTTCGTTGACGGCGTTCAAACTGGAATTTGGCTTGGACAAAATGTC TGCGACGGCGGCTTCTGCTATCGAAGCCAACGTTGTTTCCGTGTTCCAG GCCGGCGCCTACTTTGGGTGTCTTTTCTTCTATCCGATTGGCGAGATTTG GGGCCGTAAAATCGGTCTTCTTCTTTCCGGCTTTCTTTTGACGTTTGGTG CTGCTATTTCTTTGATTTCGAACTCGTCTCGTGGCCTTGGTGCCATATAT GCTGGAAGAGTACTAACAGGTTTGGGGATTGGCGGATGTCTGAGTTTGG CCCCAATCTACGTTTCTGAAATCGCGCCTGCAGCAATCAGAGGCAAGCT TGTGGGCTGCTGGGAAGTGTCATGGCAGGTGGGCGGCATTGTTGGCTAC TGGATCAATTACGGAGTCTTGCAGACTCTTCCGATTAGCTCACAACAAT GGATCATCCCGTTTGCTGTACAATTGATCCCATCGGGGCTTTTCTGGGGC CTTTGTCTTTTGATTCCAGAGCTGCCACGTTTTCTTGTATCGAAGGGAAA GATCGATAAGGCGCGCAAAAACTTAGCGTACTTGCGTGGACTTAGCGA GGACCACCCCTATTCTGTTTTTGAGTTGGAGAACATTAGTAAGGCCATT GAAGAGAACTTCGAGCAAACAGGAAGGGGTTTTTTCGACCCATTGAAA GCTTTGTTTTTCAGCAAAAAAATGCTTTACCGCCTTCTCTTGTCCACGTC AATGTTCATGATGCAGAATGGCTATGGAATCAATGCTGTGACATACTAC TCGCCCACGATCTTCAAATCCTTAGGCGTTCAGGGCTCAAACGCCGGTT TGCTCTCAACAGGAATTTTCGGTCTTCTTAAAGGTGCCGCTTCGGTGTTC TGGGTCTTTTTCTTGGTTGACACATTCGGCCGCCGGTTTTGTCTTTGCTA CCTCTCTCTCCCCTGCTCGATCTGCATGTGGTATATTGGCGCATACATCA AGATTGCCAACCCTTCAGCGAAGCTTGCTGCAGGAGACACAGCCACCA CCCCAGCAGGAACTGCAGCGAAAGCGATGCTTTACATATGGACGATTTT CTACGGCATTACGTGGAATGGTACGACCTGGGTGATCTGCGCGGAGATT TTCCCCCAGTCGGTGAGAACAGCCGCGCAGGCCGTCAACGCTTCTTCTA ATTGGTTCTGGGCTTTCATGATCGGCCACTTCACTGGCCAGGCGCTCGA GAATATTGGGTACGGATACTACTTCTTGTTTGCGGCGTGCTCTGCAATCT TCCCTGTGGTAGTCTGGTTTGTGTACCCCGAAACAAAGGGTGTGCCTTT GGAGGCCGTGGAGTATTTGTTCGAGGTGCGTCCTTGGAAAGCGCACTCA TATGCTTTGGAGAAGTACCAGATTGAGTACAACGAGGGTGAATTCCACC

		AACATAAGCCCGAAGTACTCTTACAAGGGTCTGAAAACTCGGACACGA GCGAGAAAAGCCTCGCCTGA
25	Nucleic acid sequence of *H0 Metschnikowia species QUP2* codon optimized for expression in *S. cerevisiae*	ATGGGTTTCAGAAACTTGAAGAGAAGATTGTCTAACGTTGGTGACTCCA TGTCTGTTCACTCTGTTAAGGAAGAAGAAGACTTCTCCAGAGTTGAAAT CCCAGATGAAATCTACAACTACAAGATCGTCTTGGTTGCTTTGACTGCT GCTTCTGCTGCTATCATCATCGGTTACGATGCTGGTTTCATTGGTGGTAC TGTTTCTTTGACTGCTTTCAAGTCTGAATTCGGTTTGGACAAGATGTCTG CTACTGCTGCTTCTGCTATCGAAATGGGTTTCAGAAACTTGAAGAGGCG TTTGTCTAATGTTGGTGATTCCATGTCTGTTCACTCCGTCAAAGAAGAAG AGGATTTCTCCAGAGTTGAAATCCCAGACGAAATCTACAACTACAAGAT CGTTTTGGTTGCTTTGACTGCTGCTTCTGCTGCTATTATCATTGGTTATG ATGCTGGTTTCATCGGTGGTACTGTTTCTTTGACAGCTTTCAAGTCTGAA TTCGGTTTGGATAAGATGTCTGCTACAGCTGCTTCAGCTATTGAAGCTA ATGTTGTCTCTGTTTTTCAAGCTGGTGCTTACTTTGGTTGCCTGTTTTTTT ACCCAATTGGTGAAATTTGGGGTCGTAAGATTGGTTTGTTGTTGTCTGGT TTCTTGTTGACTTTTGGTGCTGCCATTTCCTTGATCTCTAATTCTTCTAGA GGTTTGGGTGCTATCTATGCTGGTAGAGTTTTGACTGGTTTAGGTATTGG TGGTTGTTCTTCTTTAGCTCCCATCTACGTTAGTGAAATTGCTCCAGCTG CAATTAGAGGTAAGTTAGTTGGTTGTTGGGAAGTTTCTTGGCAAGTTGG TGGTATCGTTGGTTATTGGATTAACTATGGTGTCTTGCAAACCCTGCCAA TCTCTTCTCAACAATGGATTATTCCATTCGCCGTTCAATTGATTCCATCT GGTTTGTTTTGGGGTTTGTGCTTGTTGATTCCAGAATCTCCAAGATTCTT GGTGTCCAAAGGTAAGATTGATAAGGCCAGAAAGAACTTGGCTTACTT GAGAGGTTTGTCTGAAGATCATCCATACTCCGTTTTTGAGTTGGAGAAC ATTTCCAAGGCCATCGAAGAAAACTTTGAACAAACAGGTAGAGGTTTCT TCGACCCATTGAAGGCTTTGTTTTTCAGCAAGAAAATGCTGTACAGGCT GCTGTTGTCTACTTCTATGTTTATGATGCAAAACGGCTACGGTATTAACG CTGTTACTTATTACTCTCCCACCATCTTTAAGTCCTTGGGTGTTCAAGGT TCTAATGCCGGTTTGTTATCTACTGGTATTTTCGGTTTGTTGAAAGGTGC CGCTTCTGTTTTTTGGGTTTTCTTCTTGGTTGATACCTTCGGTAGAAGATT CTGTTTGTGCTATTTGTCTTTGCCATGCTCTATCTGCATGTGGTATATTG GTGCCTACATTAAGATTGCTAACCCATCTGCTAAATTGGCTGCTGGTGA TACTGCTACTACTCCAGCTGGTACTGCTGCTAAAGCTATGTTGTATATTT GGACCATCTTCTACGGTATCACTTGGAATGGTACTACCTGGGTTATTTGC GCTGAAATTTTTCCACAATCTGTTAGAACAGCTGCTCAAGCTGTTAATG CTTCTTCTAATTGGTTTTGGGCCTTCATGATTGGTCATTTTACTGGTCAA GCTTTGGAAAACATTGGTTACGGTTACTACTTTTTGTTCGCTGCTTGTTC CGCTATTTTCCCAGTTGTAGTTTGGTTCGTTTACCCAGAAACAAAAGGT GTTCCATTGGAAGCTGTTGAATACTTGTTTGAAGTTAGACCATGGAAGG CTCATTCTTACGCTTTAGAAAAGTACCAGATCGAGTACAACGAAGGTGA ATTCCATCAACATAAGCCAGAAGTTTTGTTGCAGGGTTCTGAAAACTCT GATACCTCTGAAAAGTCTTTGGCCTGAAACGAAGGTGAATTCCACCAAC ATAAGCCAGAAGTTTTGTTGCAAGGTTCTGAAAACTCTGACACTTCTGA AAAGTCTTTGGCTTAA
26	Nucleic acid sequence of *H0 Metschnikowia species* APS1/HGT19	ATGTCAGAAAAGCCTGTTGTGTCGCACAGCATCGACACGACGCTGTCTA CGTCATCGAAACAAGTCTATGACGGTAACTCGCTTCTTAAGACCCTGAA TGAGCGCGATGGCGAACGCGGCAATATCTTGTCGCAGTACACTGAGGA ACAGGCCATGCAAATGGGCCGCAACTATGCGTTGAAGCACAATTTAGA TGCGACACTCTTTGGAAAGGCGGCCGCGGTCGCAAGAAACCCATACGA GTTCAATTCGATGAGTTTTTTGACCGAAGAGGAAAAAGTCGCGCTTAAC ACGGAGCAGACCAAGAAATGGCACATCCCAAGAAAGTTGGTGGAGGTG ATTGCATTGGGGTCCATGGCCGCTGCGGTGCAGGGTATGGATGAGTCGG TGGTGAATGGTGCAACGCTTTTCTACCCCACGGCAATGGGTATCACAGA TATCAAGAATGCCGATTTGATTGAAGGTTTGATCAACGGTGCGCCCTAT CTTTGCTGCGCCATCATGTGCTGGACATCTGATTACTGGAACAGGAAGT TGGGCCGTAAGTGGACCATTTTCTGGACATGTGCCATTTCTGCAATCAC ATGTATCTGGCAAGGTCTCGTCAATTTGAAATGGTACCATTTGTTCATTG

		CGCGTTTCTGCTTGGGTTTCGGTATCGGTGTCAAGTCTGCCACCGTGCCT GCGTATGCTGCCGAAACCACCCCGGCCAAAATCAGAGGCTCGTTGGTCA TGCTTTGGCAGTTCTTCACCGCTGTCGGAATCATGCTTGGTTACGTGGCG TCTTTGGCATTCTATTACATTGGTGACAATGGCATTTCTGGCGGCTTGAA CTGGAGATTGATGCTAGGATCTGCATGTCTTCCAGCTATCGTTGTGTTAG TCCAAGTTCCGTTTGTTCCAGAATCCCCTCGTTGGCTCATGGGTAAGGA AAGACACGCTGAAGCATATGATTCGCTCCGGCAATTGCGGTTCAGTGAA ATCGAGGCGGCCCGTGACTGTTTCTACCAGTACGTGTTGTTGAAAGAGG AGGGCTCTTATGGAACGCAGCCATTCTTCAGCAGAATCAAGGAGATGTT CACCGTGAGAAGAAACAGAAATGGTGCATTGGGCGCGTGGATCGTCAT GTTCATGCAGCAGTTCTGTGGAATCAACGTCATTGCTTACTACTCGTCGT CGATCTTCGTGGAGTCGAATCTTTCTGAGATCAAGGCCATGTTGGCGTC TTGGGGGTTCGGTATGATCAATTTCTTGTTTGCAATTCCAGCGTTCTACA CCATTGACACGTTTGGCCGACGCAACTTGTTGCTCACTACTTTCCCTCTT ATGGCGGTATTCTTACTCATGGCCGGATTCGGGTTCTGGATCCCGTTCG AGACAAACCCACACGGCCGTTTGGCGGTGATCACTATTGGTATCTATTT GTTTGCATGTGTCTACTCTGCGGGCGAGGGACCAGTTCCCTTCACATAC TCTGCCGAAGCATTCCCGTTGTATATCCGTGACTTGGGTATGGGCTTTGC CACGGCCACGTGTTGGTTCTTCAACTTCATTTTGGCATTTTCCTGGCCTA GAATGAAGAATGCATTCAAGCCTCAAGGTGCCTTTGGCTGGTATGCCGC CTGGAACATTGTTGGCTTCTTCTTAGTGTTATGGTTCTTGCCCGAGACAA AGGGCTTGACGTTGGAGGAATTGGACGAAGTGTTTGATGTGCCTTTGAG AAAACACGCGCACTACCGTACCAAAGAATTAGTATACAACTTGCGCAA ATACTTCTTGAGGCAGAACCCTAAGCCATTGCCGCCACTTTATGCACAC CAAAGAATGGCTGTTACCAACCCAGAATGGTTGGAAAAGACCGAGGTC ACGCACGAGGAGAATATCTAG
27	Nucleic acid sequence of *H0 Metschnikowia species APS1/HGT19* codon optimized for expression in *S. cerevisiae*	ATGTCTGAAAAGCCAGTTGTTTCTCACTCTATCGACACCACCTCTTCTAC CTCTTCTAAGCAAGTCTACGACGGTAACTCTTTGTTGAAGACCTCTAAC GAAAGAGACGGTGAAAGAGGTAACATCTTGTCTCAATACACTGAAGAA CAAGCAATGCAAATGGGTAGAAACTACGCTTTGAAGCACAACTTGGAC GCTACCTTGTTCGGTAAGGCTGCTGCTGTCGCTAGAAACCCATACGAGT TCAACTCTATGTCTTTCTTGACCGAAGAAGAAAAGGTCGCTTTGAACAC CGAACAAACCAAGAAGTGGCACATCCCAAGAAAGTTGGTTGAAGTTAT TGCTTTGGGTTCTATGGCTGCTGCTGTTCAAGGTATGGACGAATCTGTTG TTAACGGTGCTACCTTGTTCTACCCAACCGCTATGGGTATCACCGACAT CAAGAACGCTGACTTGATTGAAGGTTTGATTAACGGTGCCCCATACTTG TGTTGTGCTATTATGTGTTGGACCTCTGACTACTGGAACAGAAAGTTGG GTAGAAAGTGGACCATTTTCTGGACCTGTGCTATTTCTGCTATCACCTGT ATCTGGCAAGGTTTGGTCAACTTGAAGTGGTATCACTTGTTCATTGCTA GATTCTGTTTGGGTTTCGGTATCGGTGTCAAGTCTGCTACCGTTCCAGCC TACGCTGCTGAAACCACCCCAGCCAAGATTAGAGGTTCTTTGGTTATGT TGTGGCAATTCTTCACCGCTGTCGGTATTATGTTGGGTTACGTTGCTTCT TTGGCTTTCTACTACATTGGTGACAACGGTATTTCTGGTGGTTTGAACTG GAGATTGATGTTGGGTTCTGCTTGTTTGCCAGCCATCGTTGTTTTGGTCC AAGTTCCATTCGTTCCAGAATCTCCAAGATGGTTGATGGGTAAGGAAAG ACACGCTGAAGCCTACGACTCTTTGAGACAATTGAGATTCTCTGAAATC GAAGCCGCTAGAGACTGTTTCTACCAATACGTTTTGTTGAAGGAAGAAG GTTCTTACGGTACTCAACCATTCTTCTCTAGAATCAAGGAAATGTTCACC GTTAGAAGAAACAGAAACGGTGCTTTGGGTGCTTGGATTGTTATGTTTA TGCAACAATTCTGTGGTATCAACGTCATTGCTTACTACTCTTCTTCTATC TTCGTTGAATCTAACTTGTCTGAAATCAAGGCTATGTTGGCTTCTTGGGG TTTCGGTATGATTAACTTCTTGTTCGCTATTCCAGCCTTCTACACCATTG ACACCTTCGGTAGAAGAAACTTGTTGTTGACTACTTTCCCATTGATGGCT GTTTTCTTGTTGATGGCTGGTTTCGGTTTCTGGATTCCATTCGAAACCAA CCCACACGGTAGATTGGCTGTTATCACTATTGGTATCTACTTGTTCGCTT GTGTCTACTCTGCTGGTGAAGGTCCAGTTCCATTCACCTACTCTGCTGAA GCCTTCCCATTGTACATCAGAGACTTGGGTATGGGTTTCGCTACCGCTA CCTGTTGGTTCTTCAACTTCATTTTGGCTTTCTCTTGGCCAAGAATGAAG

		AACGCTTTCAAGCCTCAAGGTGCTTTCGGTTGGTACGCTGCTTGGAACATTGTTGGTTTCTTCTTGGTTTTGTGGTTCTTGCCAGAAACTAAGGGTTTGACTTTGGAAGAATTGGACGAAGTTTTCGACGTTCCATTGAGAAAGCACGCTCACTACAGAACTAAGGAATTGGTTTACAACTTGAGAAAGTACTTCTTGAGACAAAACCCAAAGCCATTGCCACCATTGTACGCTCACCAAAGAATGGCTGTTACCAACCCAGAATGGTTGGAAAAGACCGAAGTCACCCACGAAGAAAACATCTAA
44	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Aps1p/Hgt19 codon optimized for expression in *S. cerevisiae* (with K4R; K20R; K30R and K93R mutations)	MSERPVVSHSIDTTSSTSSRQVYDGNSLLRTSNERDGERGNILSQYTEEQAMQMGRNYALKHNLDATLFGKAAAVARNPYEFNSMSFLTEEERVALNTEQTKKWHIPRKLVEVIALGSMAAAVQGMDESVVNGATLFYPTAMGITDIKNADLIEGLINGAPYLCCAIMCWTSDYWNRKLGRKWTIFWTCAISAITCIWQGLVNLKWYHLFIARFCLGFGIGVKSATVPAYAAETTPAKIRGSLVMLWQFFTAVGIMLGYVASLAFYYIGDNGISGGLNWRLMLGSACLPAIVVLVQVPFVPESPRWLMGKERHAEAYDSLRQLRFSEIEAARDCFYQYVLLKEEGSYGTQPFFSRIKEMFTVRRNRNGALGAWIVMFMQQFCGINVIAYYSSSIFVESNLSEIKAMLASWGFGMINFLFAIPAFYTIDTFGRRNLLLTTFPLMAVFLLMAGFGFWIPFETNPHGRLAVITIGIYLFACVYSAGEGPVPFTYSAEAFPLYIRDLGMGFATATCWFFNFILAFSWPRMKNAFKPQGAFGWYAAWNIVGFFLVLWFLPETKGLTLEELDEVFDVPLRKHAHYRTKELVYNLRKYFLRQNPKPLPPLYAHQRMAVTNPEWLEKTEVTHEENI
45	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Hxt5p (with K7R; K10R, K29R; K43R and K58R mutations)	MSIFEGRDGRGVSSTESLSNDVRYDNMERVDQDVLRHNFNFDREFEELEIEAAQVNDRPSFVDRILSLEYKLHFENKNHMVWLLGAFAAAAGLLSGLDQSIISGASIGMNKALNLTEREASLVSSLMPLGAMAGSMIMTPLNEWFGRKSSLIISCIWYTIGSALCAGARDHHMMYAGRFILGVGVGIEGGCVGIYISESVPANVRGSIVSMYQFNIALGEVLGYAVAAIFYTVHGGWRFMVGSSLVFSTILFAGLFFLPESPRWLVHKGRNGMAYDVWKRLRDINDESAKLEFLEMRQAAYQERERRSQESLFSSWGELFTIARNRRALTYSVIMITLGQLTGVNAVMYYMSTLMGAIGFNEKDSVFMSLVGGGSLLIGTIPAILWMDRFGRRVWGYNLVGFFVGLVLVGVGYRFNPVTQKAASEGVYLTGLIVYFLFFGSYSTLTWVIPSESFDLRTRSLGMTICSTFLYLWSFTVTYNFTKMSAAFTYTGLTLGFYGGIAFLGLIYQVCFMPETKDKTLEEIDDIFNRSAFSIARENISNLKKGIW
46	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Gxf2p/Gal2p (with K23R, K26R, K35R, K542R and K546R mutations)	MSAEQEQQVSGTSATIDGLASLRQERTAEEEDAFRPKPATAYFFISFLCGLVAFGGYVFGFDTGTISGFVNMDDYLMRFGQQHADGTYYLSNVRTGLIVSIFNIGCAVGGLALSKVGDIWGRRIGIMVAMIIYMVGIIIQIASQDKWYQYFIGRLITGLGVGTTSVLSPLFISESAPKHLRGTLVCCFQLMVTLGIFLGYCTTYGTKNYTDSRQWRIPLGLCFAWALLLISGMVFMPESPRFLIERQRFDEAKASVAKSNQVSTEDPAVYTEVELIQAGIDREALAGSAGWKELITGKPKMLQRVILGMMLQSIQQLTGNNYFFYYGTTIFKAVGMSDSFQTSIVLGIVNFASTFVGIWAIERMGRRSCLLVGSACMSVCFLIYSILGSVNLYIDGYENTPSNTRKPTGNAMIFITCLFIFFFASTWAGGVYSIVSETYPLRIRSKGMAVATAANWMWGFLISFFTPFITSAIHFYYGFVFTGCLIFSFFYVFFFVRETKGLSLEEVDELYATDLPPWKTAGWTPPSAEDMAHTTGFAEAARPTNRHV
47	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Gxf1p (with K9R and K24R mutations)	MSQDELHTRSGVETPINDSLLEERHDVTPLAALPEKSFKDYISISIFCLFVAFGGFVFGFDTGTISGFVNMSDFKTRFGEMNAQGEYYLSNVRTGLMVSIFNVGCAVGGIFLCKIADVYGRRIGLMFSMVVYVVGIIIQIASTTKWYQYFIGRLIAGLAVGTVSVISPLFISEVAPKQLRGTLVCCFQLCITLGIFLGYCTTYGTKTYTDSRQWRIPLGICFAWALFLVAGMLNMPESPRYLVEKSRIDDARKSIARSNKVSEEDPAVYTEVQLIQAGIDREALAGSATWMELVTGKPKIFRRVIMGVMLQSLQQLTGDNYFFYYGTTIFKAVGLQDSFQTSIILGIVNFASTFVGIYAIERMGRRLCLLTGSACMFVCFIIYSLIGTQHLYKNGFSNEPSNTYKPSGNAMIFITCLYIFFFASTWAGGVYCIVSESYPLRIRSKAMSVATAANWMWGFLISFFTPFITSAIHFYYGFVFTGCLAFSFFYVYFFVVETKGLSLEEVDILYASGTLPWKSSGWVPPTADEMAHNAFDNKPTDEQV

48	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Xyt1p (with K6R and S75L mutations)	MGYEERLVAPALKFKNFLDKTPNIHNVYVIAAISCTSGMMFGFDISSMSVF VDQQPYLKMFDNPSSVIQGFITALMSLGSFFGSLTSTFISEPFGRRASLFICGI LWVIGAAVQSSSQNRAQLICGRIIAGWGIGFGSSVAPVYGSEMAPRKIRGTI GGIFQFSVTVGIFIMFLIGYGCSFIQGKASFRIPWGVQMVPGLILLIGLFFIPES PRWLAKQGYWEDAEIIVANVQAKGNRNDANVQIEMSEIKDQLMLDEHLK EFTYADLFTKKYRQRTITAIFAQIWQQLTGMNVMMYYIVYIFQMAGYSGN TNLVPSLIQYIINMAVTVPALFCLDLLGRRTILLAGAAFMMAWQFGVAGIL ATYSEPAYISDTVRITIPDDHKSAAKGVIACCYLFVCSFAFSWGVGIWVYCS EVWGDSQSRQRGAALATSANWIFNFAIAMFTPSSFKNITWKTYIIYATFCAC MFIHVFFFFPETKGKRLEEIGQLWDEGVPAWRSAKWQPTVPLASDAELAH KMDVAHAEHADLLATHSPSSDEKTGTV
49	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species APS1/HGT19* (with K4R; K20R; K30R and K93R mutations)	ATGTCTGAAAGACCAGTTGTTTCTCACTCTATCGACACCACCTCTTCTAC CTCTTCTAGACAAGTCTACGACGGTAACTCTTTGTTGAGGACCTCTAAC GAAAGAGACGGTGAAAGAGGTAACATCTTGTCTCAATACACTGAAGAA CAAGCAATGCAAATGGGTAGAAACTACGCTTTGAAGCACAACTTGGAC GCTACCTTGTTCGGTAAGGCTGCTGCTGTCGCTAGAAACCCATACGAGT TCAACTCTATGTCTTTCTTGACCGAAGAAGAAAGAGTCGCTTTGAACAC CGAACAAACCAAGAAGTGGCACATCCCAAGAAAGTTGGTTGAAGTTAT TGCTTTGGGTTCTATGGCTGCTGCTGTTCAAGGTATGGACGAATCTGTTG TTAACGGTGCTACCTTGTTCTACCCAACCGCTATGGGTATCACCGACAT CAAGAACGCTGACTTGATTGAAGGTTTGATTAACGGTGCCCCATACTTG TGTTGTGCTATTATGTGTTGGACCTCTGACTACTGGAACAGAAAGTTGG GTAGAAAGTGGACCATTTTCTGGACCTGTGCTATTTCTGCTATCACCTGT ATCTGGCAAGGTTTGGTCAACTTGAAGTGGTATCACTTGTTCATTGCTA GATTCTGTTTGGGTTTCGGTATCGGTGTCAAGTCTGCTACCGTTCCAGCC TACGCTGCTGAAACCACCCCAGCCAAGATTAGAGGTTCTTTGGTTATGT TGTGGCAATTCTTCACCGCTGTCGGTATTATGTTGGGTTACGTTGCTTCT TTGGCTTTCTACTACATTGGTGACAACGGTATTTCTGGTGGTTTGAACTG GAGATTGATGTTGGGTTCTGCTTGTTTGCCAGCCATCGTTGTTTTGGTCC AAGTTCCATTCGTTCCAGAATCTCCAAGATGGTTGATGGGTAAGGAAAG ACACGCTGAAGCCTACGACTCTTTGAGACAATTGAGATTCTCTGAAATC GAAGCCGCTAGAGACTGTTTCTACCAATACGTTTTGTTGAAGGAAGAAG GTTCTTACGGTACTCAACCATTCTTCTCTAGAATCAAGGAAATGTTCACC GTTAGAAGAAACAGAAACGGTGCTTTGGGTGCTTGGATTGTTATGTTTA TGCAACAATTCTGTGGTATCAACGTCATTGCTTACTACTCTTCTTCTATC TTCGTTGAATCTAACTTGTCTGAAATCAAGGCTATGTTGGCTTCTTGGGG TTTCGGTATGATTAACTTCTTGTTCGCTATTCCAGCCTTCTACACCATTG ACACCTTCGGTAGAAGAAACTTGTTGTTGACTACTTTCCCATTGATGGCT GTTTTCTTGTTGATGGCTGGTTTCGGTTTCTGGATTCCATTCGAAACCAA CCCACACGGTAGATTGGCTGTTATCACTATTGGTATCTACTTGTTCGCTT GTGTCTACTCTGCTGGTGAAGGTCCAGTTCCATTCACCTACTCTGCTGAA GCCTTCCCATTGTACATCAGAGACTTGGGTATGGGTTTCGCTACCGCTA CCTGTTGGTTCTTCAACTTCATTTTGGCTTTCTCTTGGCCAAGAATGAAG AACGCTTTCAAGCCTCAAGGTGCTTTCGGTTGGTACGCTGCTTGGAACA TTGTTGGTTTCTTCTTGGTTTTGTGGTTCTTGCCAGAAACTAAGGGTTTG ACTTTGGAAGAATTGGACGAAGTTTTCGACGTTCCATTGAGAAAGCACG CTCACTACAGAACTAAGGAATTGGTTTACAACTTGAGAAAGTACTTCTT GAGACAAAACCCAAAGCCATTGCCACCATTGTACGCTCACCAAAGAAT GGCTGTTACCAACCCAGAATGGTTGGAAAAGACCGAAGTCACCCACGA AGAAAACATCTAA
50	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species HXT5* (with K7R; K10R,	ATGTCCATTTTCGAAGGTAGGGATGGTAGAGGTGTTTCCTCTACTGAAT CCTTGTCTAACGATGTTAGATACGACAACATGGAAAGAGTTGACCAAG ATGTTTTGAGGCACAATTTCAACTTCGACAGAGAGTTCGAAGAATTGGA AATTGAAGCTGCCCAAGTTAACGATAGACCATCTTTCGTTGATAGGATC TTGTCTTTGGAGTACAAGTTGCACTTCGAAAACAAGAATCACATGGTTT GGTTGTTGGGTGCTTTTGCTGCTGCTGCAGGTTTGTTGTCTGGTTTGGAT CAATCTATTATTTCCGGTGCCTCTATCGGTATGAACAAGGCTTTGAATTT

	K29R; K43R and K58R mutations)	GACCGAAAGAGAAGCCTCTTTGGTCAGTTCTTTGATGCCATTGGGTGCT ATGGCTGGTTCTATGATTATGACTCCATTGAATGAATGGTTCGGCCGTA AATCCTCCTTGATTATTTCTTGTATTTGGTACACCATCGGTTCTGCTTTGT GTGCTGGTGCTAGAGATCATCACATGATGTATGCTGGTAGATTCATCTT AGGTGTTGGTGTTGGTATTGAAGGTGGTTGCGTTGGTATCTACATTTCTG AATCTGTTCCAGCCAATGTCAGAGGTTCTATCGTTTCTATGTACCAGTTC AACATTGCCTTGGGTGAAGTTTTGGGTTATGCTGTTGCTGCTATTTTCTA CACTGTTCATGGTGGTTGGAGGTTTATGGTTGGTTCTTCTTTGGTTTTCT CCACCATTTTGTTTGCCGGCTTGTTTTTTTTGCCAGAATCTCCAAGATGG TTGGTCCATAAGGGTAGAAATGGTATGGCTTACGATGTTTGGAAGAGAT TGAGAGATATCAACGATGAATCCGCCAAGTTGGAATTCTTGGAAATGA GACAAGCTGCCTACCAAGAAAGAGAAAGAAGATCTCAAGAGTCCTTGT TTTCTTCATGGGGTGAGTTGTTTACCATTGCTAGAAATAGAAGGGCTTT GACCTACTCCGTTATTATGATTACTTTGGGTCAGTTGACTGGTGTTAACG CTGTTATGTATTACATGTCTACTTTGATGGGTGCCATCGGTTTTAACGAA AAGGATTCTGTTTTCATGTCCTTGGTTGGTGGTGGTTCTTTGTTGATTGG TACTATTCCAGCTATCTTGTGGATGGATAGATTCGGTAGAAGAGTTTGG GGTTACAATTTGGTTGGTTTTTTCGTCGGTTTGGTATTGGTCGGTGTTGG TTATAGATTCAACCCAGTTACTCAAAAGGCTGCTTCTGAAGGTGTTTATT TGACTGGTTTGATCGTCTACTTCTTGTTCTTCGGTTCTTACTCTACATTGA CCTGGGTTATTCCATCCGAATCTTTCGATTTGAGAACCAGATCTTTGGGT ATGACCATTTGCTCTACTTTCTTGTACTTGTGGTCTTTCACTGTCACTTAC AACTTCACTAAGATGTCTGCTGCTTTCACTTACACAGGTTTGACTTTGGG TTTTTACGGTGGTATTGCTTTCTTGGGTTTGATCTACCAAGTTTGCTTTAT GCCAGAAACTAAGGACAAGACCTTGGAAGAAATCGATGACATCTTTAA CAGATCCGCTTTCTCTATTGCCAGGGAAAACATTAGCAACTTGAAGAAA GGTATCTGGTAA
51	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species GXF2/GAL2* (with K23R, K26R, K35R, K542R and K546R mutations)	ATGTCCGCTGAACAAGAACAACAAGTTTCTGGTACTTCTGCCACTATTG ATGGTTTGGCTTCTTTGAGGCAAGAAAGGACTGCTGAAGAAGAAGATG CTTTTAGGCCAAAACCAGCTACTGCCTACTTCTTCATTTCTTTCTTGTGT GGTTTGGTTGCTTTCGGTGGTTACGTTTTTGGTTTTGATACCGGTACTAT CTCCGGTTTCGTTAACATGGATGATTACTTGATGAGATTCGGTCAACAA CATGCTGATGGTACTTACTACTTGTCCAATGTTAGAACCGGTTTGATCGT CAGTATTTTCAACATTGGTTGTGCTGTTGGTGGTTTGGCATTGTCTAAAG TTGGTGATATTTGGGGTAGAAGAATCGGTATTATGGTTGCCATGATCAT CTACATGGTTGGTATCATTATTCAAATCGCCTCCCAAGACAAGTGGTAT CAATACTTTATTGGTAGATTGATCACCGGTTTGGGTGTTGGTACTACTTC TGTTTTGTCTCCTTTGTTCATTTCCGAATCCGCTCCAAAACATTTGAGAG GTACTTTGGTTTGCTGCTTCCAATTGATGGTAACCTTGGGTATTTTCTTG GGTTACTGTACTACTTACGGTACTAAGAACTACACCGATTCTAGACAAT GGAGAATTCCATTGGGTTTGTGTTTTGCTTGGGCCTTGTTGTTGATTTCT GGTATGGTTTTTATGCCAGAATCCCCAAGATTCTTGATCGAAAGACAAA GATTCGATGAAGCTAAGGCTTCTGTTGCCAAGTCTAATCAAGTTTCTAC TGAAGATCCAGCCGTTTACACTGAAGTTGAATTGATTCAAGCCGGTATT GATAGAGAAGCTTTGGCTGGTTCTGCTGGTTGGAAAGAATTGATTACTG GTAAGCCAAAGATGTTGCAAAGAGTCATTTTGGGTATGATGTTACAATC CATCCAACAATTGACCGGTAACAATTACTTCTTCTACTACGGTACAACC ATCTTCAAAGCTGTTGGTATGTCCGATTCTTTTCAAACCTCTATAGTCTT GGGTATCGTTAACTTCGCTTCTACCTTTGTTGGTATTTGGGCCATTGAAA GAATGGGTAGAAGATCTTGTTTGTTGGTTGGTTCAGCTTGTATGTCTGTT TGCTTCTTGATCTACTCTATCTTGGGTTCAGTCAACTTGTACATCGATGG TTACGAAAACACTCCATCTAACACTAGAAAGCCAACTGGTAACGCCATG ATTTTCATTACCTGTTTGTTCATCTTTTTCTTCGCCTCTACTTGGGCTGGT GGTGTTTATTCTATAGTTTCTGAAACCTACCCATTGAGAATCAGATCTAA AGGTATGGCTGTTGCTACTGCTGCTAATTGGATGTGGGGTTTTTTGATCT CTTTCTTTACCCCATTCATCACCTCCGCTATTCATTTTTACTACGGTTTTG TTTTCACCGGTTGCTTGATCTTCTCATTCTTTTACGTATTCTTTTTCGTCC GTGAAACTAAGGGTTTGTCCTTGGAAGAAGTTGACGAATTATACGCTAC

		TGATTTGCCACCATGGAAAACTGCAGGTTGGACTCCACCATCAGCTGAA GATATGGCTCATACAACTGGTTTTGCTGAAGCTGCTAGGCCTACAAACA GACACGTTTGA
52	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species GXF1* (with K9R and K24R mutations)	ATGTCTCAAGATGAATTGCACACCAGATCTGGTGTTGAAACTCCAATCA ACGACTCCTTGTTGGAAGAAAGACATGATGTTACTCCATTGGCTGCTTT GCCAGAAAAATCTTTCAAGGACTACATCTCCATCTCCATTTTCTGTTTGT TTGTTGCTTTCGGTGGTTTCGTTTTCGGTTTTGATACTGGTACTATTTCCG GTTTCGTTAACATGTCTGATTTCAAGACTAGGTTCGGTGAAATGAATGC TCAGGGTGAATATTACTTGTCCAACGTTAGAACTGGCCTGATGGTTTCT ATTTTCAATGTTGGTTGTGCTGTCGGTGGTATTTTCTTGTGTAAAATTGC TGATGTCTACGGTAGAAGGATCGGTTTGATGTTTTCTATGGTTGTCTACG TTGTCGGTATCATTATTCAAATTGCTTCTACCACCAAGTGGTATCAGTAC TTCATTGGTAGATTGATTGCTGGTTTGGCTGTTGGTACTGTTTCTGTTAT TTCCCCTTTGTTCATTTCCGAAGTTGCTCCAAAACAATTGAGAGGTACTT TGGTTTGCTGTTTCCAATTGTGTATTACCTTGGGTATCTTCTTGGGTTACT GTACTACTTACGGTACTAAGACTTACACCGATTCTAGACAATGGCGTAT TCCATTGGGTATTTGTTTTGCTTGGGCTTTGTTTTTGGTTGCCGGTATGTT GAATATGCCAGAATCTCCAAGATACTTGGTCGAAAAGTCCAGAATTGAT GATGCCAGAAAGTCCATTGCTAGGTCTAACAAAGTTTCCGAAGAAGATC CAGCTGTTTACACCGAAGTTCAATTGATTCAAGCCGGTATTGATAGAGA AGCTTTGGCTGGTTCTGCTACTTGGATGGAATTGGTTACTGGTAAGCCT AAGATCTTTAGAAGAGTTATCATGGGTGTCATGTTGCAATCCTTGCAAC AATTGACTGGTGACAACTACTTTTTCTACTACGGTACAACCATTTTCAAG GCTGTCGGTTTACAAGATTCTTTCCAAACCTCCATCATTTTGGGTATCGT TAACTTCGCTTCTACCTTCGTTGGTATCTACGCTATTGAAAGAATGGGTA GAAGATTGTGTTTGTTGACAGGTTCTGCTTGTATGTTCGTTTGCTTCATC ATCTACTCATTGATCGGTACTCAGCACTTGTACAAAAACGGTTTTTCTAA CGAACCCTCCAACACTTACAAACCATCTGGTAATGCCATGATCTTCATT ACCTGCCTGTACATTTTCTTTTTCGCTTCAACTTGGGCTGGTGGTGTTTA CTGTATAGTTTCTGAATCTTACCCACTGAGGATCAGATCTAAAGCTATG TCTGTTGCTACTGCTGCAAATTGGATGTGGGGTTTTTTGATTTCTTTCTTT ACCCCATTCATCACCTCCGCTATCCATTTTTACTATGGTTTTGTTTTCACC GGTTGCTTGGCTTTCTCTTTCTTTTACGTTTACTTCTTCGTCGTCGAGACT AAGGGTTTGTCTTTGGAAGAGGTTGATATCTTGTATGCCTCTGGTACTTT GCCATGGAAATCTTCAGGTTGGGTTCCACCAACTGCTGACGAAATGGCT CATAATGCTTTTGATAACAAACCAACCGATGAACAGGTTTAA
53	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species XYT1* (with K6R and S75L mutations)	ATGGGATACGAAGAGAGATTAGTGGCCCCCGCTTTGAAATTTAAGAACT TTTTGGATAAGACCCCAAATATACATAACGTTTACGTAATTGCGGCGAT CTCGTGTACCTCAGGTATGATGTTCGGTTTCGATATATCGTCGATGTCCG TGTTCGTGGACCAACAGCCGTATTTAAAAATGTTTGATAACCCTAGCAG CGTGATACAAGGGTTTATAACTGCGTTGATGTCTTTGGGGAGCTTTTTCG GATCGCTAACGTCCACTTTTATTTCAGAACCTTTTGGTAGACGTGCCTCT TTGTTCATATGCGGGATCCTTTGGGTAATTGGGGCGGCAGTTCAAAGTT CTTCTCAGAACCGTGCGCAGCTTATTTGTGGCCGAATTATTGCAGGGTG GGGCATCGGATTCGGTTCTAGCGTTGCGCCGGTATACGGTTCAGAAATG GCCCCACGCAAAATTAGAGGAACAATCGGAGGTATTTTTCAATTTTCTG TCACGGTCGGAATATTCATAATGTTCCTGATTGGCTACGGCTGCTCATTT ATACAAGGCAAGGCCAGTTTTAGAATTCCGTGGGGAGTTCAAATGGTAC CAGGTCTCATTCTGTTGATCGGACTATTCTTCATTCCTGAATCCCCAAGA TGGTTAGCCAAACAAGGCTACTGGGAAGACGCTGAGATCATCGTAGCA AACGTTCAAGCTAAGGGTAACAGGAACGATGCTAATGTGCAAATTGAA ATGTCCGAGATAAAAGATCAGTTAATGCTTGACGAGCATTTAAAGGAGT TTACTTATGCCGATTTGTTTACCAAAAAATACCGGCAAAGGACGATAAC AGCTATATTTGCCCAAATATGGCAACAGCTGACAGGTATGAATGTCATG ATGTACTACATCGTATATATATTTCAAATGGCAGGTTATTCAGGTAATA CTAATTTAGTTCCTTCACTCATTCAGTATATTATAAATATGGCTGTTACG GTCCCCGCATTGTTCTGTCTTGATCTGCTTGGCAGGAGGACAATTTTATT

		AGCTGGCGCCGCTTTTATGATGGCCTGGCAATTTGGTGTTGCTGGCATTT TAGCTACTTATTCAGAGCCAGCCTATATTTCAGATACCGTGAGAATTAC AATTCCAGATGACCATAAAAGTGCCGCTAAGGGTGTCATCGCTTGCTGC TATTTGTTTGTTTGTTCCTTCGCCTTTTCCTGGGGTGTAGGTATCTGGGTT TATTGTTCAGAAGTGTGGGGTGATAGTCAATCCAGACAAAGAGGTGCTG CATTGGCAACTTCTGCTAATTGGATCTTCAATTTCGCAATTGCAATGTTT ACACCTTCTTCTTTCAAAAATATCACTTGGAAGACTTATATCATTTATGC TACATTTTGTGCTTGTATGTTCATTCATGTTTTTTTTTTTTTCCCTGAAAC AAAGGGTAAGAGACTAGAAGAAATTGGACAGCTATGGGATGAAGGTGT CCCAGCATGGAGATCTGCAAAATGGCAACCCACTGTCCCACTAGCAAGT GACGCTGAATTAGCTCACAAAATGGATGTTGCACACGCTGAACACGCA GACTTATTGGCAACCCATTCTCCAAGTAGTGACGAAAAAACTGGTACCG TTTAA
54	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Gxf1p (with K9R; K24R, K538R mutations)	MSQDELHTRSGVETPINDSLLEERHDVTPLAALPEKSFKDYISISIFCLFVAF GGFVFGFDTGTISGFVNMSDFKTRFGEMNAQGEYYLSNVRTGLMVSIFNV GCAVGGIFLCKIADVYGRRIGLMFSMVVYVVGIIIQIASTTKWYQYFIGRLI AGLAVGTVSVISPLFISEVAPKQLRGTLVCCFQLCITLGIFLGYCTTYGTKTY TDSRQWRIPLGICFAWALFLVAGMLNMPESPRYLVEKSRIDDARKSIARSN KVSEEDPAVYTEVQLIQAGIDREALAGSATWMELVTGKPKIFRRVIMGVM LQSLQQLTGDNYFFYYGTTIFKAVGLQDSFQTSIILGIVNFASTFVGIYAIER MGRRLCLLTGSACMFVCFIIYSLIGTQHLYKNGFSNEPSNTYKPSGNAMIFIT CLYIFFFASTWAGGVYCIVSESYPLRIRSKAMSVATAANWMWGFLISFFTPF ITSAIHFYYGFVFTGCLAFSFFYVYFFVVETKGLSLEEVDILYASGTLPWKSS GWVPPTADEMAHNAFDNRPTDEQV
55	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Xyt1p (with K6R, S75L, K517R, K539R mutations)	MGYEERLVAPALKFKNFLDKTPNIHNVYVIAAISCTSGMMFGFDISSMSVF VDQQPYLKMFDNPSSVIQGFITALMSLGSFFGSLTSTFISEPFGRRASLFICGI LWVIGAAVQSSSQNRAQLICGRIIAGWGIGFGSSVAPVYGSEMAPRKIRGTI GGIFQFSVTVGIFIMFLIGYGCSFIQGKASFRIPWGVQMVPGLILLIGLFFIPES PRWLAKQGYWEDAEIIVANVQAKGNRNDANVQIEMSEIKDQLMLDEHLK EFTYADLFTKKYRQRTITAIFAQIWQQLTGMNVMMYYIVYIFQMAGYSGN TNLVPSLIQYIINMAVTVPALFCLDLLGRRTILLAGAAFMMAWQFGVAGIL ATYSEPAYISDTVRITIPDDHKSAAKGVIACCYLFVCSFAFSWGVGIWVYCS EVWGDSQSRQRGAALATSANWIFNFAIAMFTPSSFKNITWKTYIIYATFCAC MFIHVFFFFPETKGKRLEEIGQLWDEGVPAWRSAKWQPTVPLASDAELAH RMDVAHAEHADLLATHSPSSDERTGTV
56	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species GXF1* (with K9R; K24R, K538R mutations)	ATGTCTCAAGATGAATTGCACACCAGATCTGGTGTTGAAACTCCAATCA ACGACTCCTTGTTGGAAGAAAGACATGATGTTACTCCATTGGCTGCTTT GCCAGAAAAATCTTTCAAGGACTACATCTCCATCTCCATTTTCTGTTTGT TTGTTGCTTTCGGTGGTTTCGTTTTCGGTTTTGATACTGGTACTATTTCCG GTTTCGTTAACATGTCTGATTTCAAGACTAGGTTCGGTGAAATGAATGC TCAGGGTGAATATTACTTGTCCAACGTTAGAACTGGCCTGATGGTTTCT ATTTTCAATGTTGGTTGTGCTGTCGGTGGTATTTTCTTGTGTAAAATTGC TGATGTCTACGGTAGAAGGATCGGTTTGATGTTTTCTATGGTTGTCTACG TTGTCGGTATCATTATTCAAATTGCTTCTACCACCAAGTGGTATCAGTAC TTCATTGGTAGATTGATTGCTGGTTTGGCTGTTGGTACTGTTTCTGTTAT TTCCCCTTTGTTCATTTCCGAAGTTGCTCCAAAACAATTGAGAGGTACTT TGGTTTGCTGTTTCCAATTGTGTATTACCTTGGGTATCTTCTTGGGTTACT GTACTACTTACGGTACTAAGACTTACACCGATTCTAGACAATGGCGTAT TCCATTGGGTATTTGTTTTGCTTGGGCTTTGTTTTTGGTTGCCGGTATGTT GAATATGCCAGAATCTCCAAGATACTTGGTCGAAAAGTCCAGAATTGAT GATGCCAGAAAGTCCATTGCTAGGTCTAACAAAGTTTCCGAAGAAGATC CAGCTGTTTACACCGAAGTTCAATTGATTCAAGCCGGTATTGATAGAGA AGCTTTGGCTGGTTCTGCTACTTGGATGGAATTGGTTACTGGTAAGCCT AAGATCTTTAGAAGAGTTATCATGGGTGTCATGTTGCAATCCTTGCAAC AATTGACTGGTGACAACTACTTTTTCTACTACGGTACAACCATTTTCAAG GCTGTCGGTTTACAAGATTCTTTCCAAACCTCCATCATTTTGGGTATCGT

		TAACTTCGCTTCTACCTTCGTTGGTATCTACGCTATTGAAAGAATGGGTA GAAGATTGTGTTTGTTGACAGGTTCTGCTTGTATGTTCGTTTGCTTCATC ATCTACTCATTGATCGGTACTCAGCACTTGTACAAAAACGGTTTTTCTAA CGAACCCTCCAACACTTACAAACCATCTGGTAATGCCATGATCTTCATT ACCTGCCTGTACATTTTCTTTTTCGCTTCAACTTGGGCTGGTGGTGTTTA CTGTATAGTTTCTGAATCTTACCCACTGAGGATCAGATCTAAAGCTATG TCTGTTGCTACTGCTGCAAATTGGATGTGGGGTTTTTTGATTTCTTTCTTT ACCCCATTCATCACCTCCGCTATCCATTTTTACTATGGTTTGTTTTCACC GGTTGCTTGGCTTTCTCTTTCTTTTACGTTTACTTCTTCGTCGTCGAGACT AAGGGTTTGTCTTTGGAAGAGGTTGATATCTTGTATGCCTCTGGTACTTT GCCATGGAAATCTTCAGGTTGGGTTCCACCAACTGCTGACGAAATGGCT CATAATGCTTTTGATAACAGACCAACCGATGAACAGGTTTAA
57	Nucleic acid sequence of ubiquitin-deficient *H0 Metschnikowia species XYT1* (with K6R, S75L, K517R, K539R mutations)	ATGGGATACGAAGAGAGATTAGTGGCCCCCGCTTTGAAATTTAAGAACT TTTTGGATAAGACCCCAAATATACATAACGTTTACGTAATTGCGGCGAT CTCGTGTACCTCAGGTATGATGTTCGGTTTCGATATATCGTCGATGTCCG TGTTCGTGGACCAACAGCCGTATTTAAAAATGTTTGATAACCCTAGCAG CGTGATACAAGGGTTTATAACTGCGTTGATGTCTTTGGGGAGCTTTTTCG GATCGCTAACGTCCACTTTTATTTCAGAACCTTTTGGTAGACGTGCCTCT TTGTTCATATGCGGGATCCTTTGGGTAATTGGGGCGGCAGTTCAAAGTT CTTCTCAGAACCGTGCGCAGCTTATTTGTGGCCGAATTATTGCAGGGTG GGGCATCGGATTCGGTTCTAGCGTTGCGCCGGTATACGGTTCAGAAATG GCCCCACGCAAAATTAGAGGAACAATCGGAGGTATTTTTCAATTTTCTG TCACGGTCGGAATATTCATAATGTTCCTGATTGGCTACGGCTGCTCATTT ATACAAGGCAAGGCCAGTTTTAGAATTCCGTGGGGAGTTCAAATGGTAC CAGGTCTCATTCTGTTGATCGGACTATTCTTCATTCCTGAATCCCCAAGA TGGTTAGCCAAACAAGGCTACTGGGAAGACGCTGAGATCATCGTAGCA AACGTTCAAGCTAAGGGTAACAGGAACGATGCTAATGTGCAAATTGAA ATGTCCGAGATAAAAGATCAGTTAATGCTTGACGAGCATTTAAAGGAGT TTACTTATGCCGATTTGTTTACCAAAAAATACCGGCAAAGGACGATAAC AGCTATATTTGCCCAAATATGGCAACAGCTGACAGGTATGAATGTCATG ATGTACTACATCGTATATATATTTCAAATGGCAGGTTATTCAGGTAATA CTAATTTAGTTCCTTCACTCATTCAGTATATTATAAATATGGCTGTTACG GTCCCCGCATTGTTCTGTCTTGATCTGCTTGGCAGGAGGACAATTTTATT AGCTGGCGCCGCTTTTATGATGGCCTGGCAATTTGGTGTTGCTGGCATTT TAGCTACTTATTCAGAGCCAGCCTATATTTCAGATACCGTGAGAATTAC AATTCCAGATGACCATAAAAGTGCCGCTAAGGGTGTCATCGCTTGCTGC TATTTGTTTGTTTGTTCCTTCGCCTTTTCCTGGGGTGTAGGTATCTGGGTT TATTGTTCAGAAGTGTGGGGTGATAGTCAATCCAGACAAAGAGGTGCTG CATTGGCAACTTCTGCTAATTGGATCTTCAATTTCGCAATTGCAATGTTT ACACCTTCTTCTTTCAAAAATATCACTTGGAAGACTTATATCATTTATGC TACATTTTGTGCTTGTATGTTCATTCATGTTTTTTTTTTTTTCCCTGAAAC AAAGGGTAAGAGACTAGAAGAAATTGGACAGCTATGGGATGAAGGTGT CCCAGCATGGAGATCTGCAAAATGGCAACCCACTGTCCCACTAGCAAGT GACGCTGAATTAGCTCACAGAATGGATGTTGCACACGCTGAACACGCA GACTTATTGGCAACCCATTCTCCAAGTAGTGACGAAAGAACTGGTACCG TTTAA
58	Amino Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Gxf2p/Gal2p (with K23R, K26R, and K35R, mutations)	MSAEQEQQVSGTSATIDGLASLRQERTAEEEDAFRPKPATAYFFISFLCGLV AFGGYVFGFDTGTISGFVNMDDYLMRFGQQHADGTYYLSNVRTGLIVSIFN IGCAVGGLALSKVGDIWGRRIGIMVAMIIYMVGIIIQIASQDKWYQYFIGRLI TGLGVGTTSVLSPLFISESAPKHLRGTLVCCFQLMVTLGIFLGYCTTYGTKN YTDSRQWRIPLGLCFAWALLLISGMVFMPESPRFLIERQRFDEAKASVAKS NQVSTEDPAVYTEVELIQAGIDREALAGSAGWKELITGKPKMLQRVILGM MLQSIQQLTGNNYFFYYGTTIFKAVGMSDSFQTSIVLGIVNFASTFVGIWAI ERMGRRSCLLVGSACMSVCFLIYSILGSVNLYIDGYENTPSNTRKPTGNAMI FITCLFIFFFASTWAGGVYSIVSETYPLRIRSKGMAVATAANWMWGFLISFF

		TPFITSAIHFYYGFVFTGCLIFSFFYVFFFVRETKGLSLEEVDELYATDLPPW KTAGWTPPSAEDMAHTTGFAEAAKPTNKHV
59	Nucleic Acid sequence of ubiquitin-deficient *H0 Metschnikowia species* Gxf2p/Gal2p (with K23R, K26R, and K35R, mutations)	ATGTCCGCTGAACAAGAACAACAAGTTTCTGGTACTTCTGCCACTATTG ATGGTTTGGCTTCTTTGAGGCAAGAAAGGACTGCTGAAGAAGAAGATG CTTTTAGGCCAAAACCAGCTACTGCCTACTTCTTCATTTCTTTCTTGTGT GGTTTGGTTGCTTTCGGTGGTTACGTTTTTGGTTTTGATACCGGTACTAT CTCCGGTTTCGTTAACATGGATGATTACTTGATGAGATTCGGTCAACAA CATGCTGATGGTACTTACTACTTGTCCAATGTTAGAACCGGTTTGATCGT CAGTATTTTCAACATTGGTTGTGCTGTTGGTGGTTTGGCATTGTCTAAAG TTGGTGATATTTGGGGTAGAAGAATCGGTATTATGGTTGCCATGATCAT CTACATGGTTGGTATCATTATTCAAATCGCCTCCCAAGACAAGTGGTAT CAATACTTTATTGGTAGATTGATCACCGGTTTGGGTGTTGGTACTACTTC TGTTTTGTCTCCTTTGTTCATTTCCGAATCCGCTCCAAAACATTTGAGAG GTACTTTGGTTTGCTGCTTCCAATTGATGGTAACCTTGGGTATTTTCTTG GGTTACTGTACTACTTACGGTACTAAGAACTACACCGATTCTAGACAAT GGAGAATTCCATTGGGTTTGTGTTTTGCTTGGGCCTTGTTGTTGATTTCT GGTATGGTTTTTATGCCAGAATCCCCAAGATTCTTGATCGAAAGACAAA GATTCGATGAAGCTAAGGCTTCTGTTGCCAAGTCTAATCAAGTTTCTAC TGAAGATCCAGCCGTTTACACTGAAGTTGAATTGATTCAAGCCGGTATT GATAGAGAAGCTTTGGCTGGTTCTGCTGGTTGGAAAGAATTGATTACTG GTAAGCCAAAGATGTTGCAAAGAGTCATTTTGGGTATGATGTTACAATC CATCCAACAATTGACCGGTAACAATTACTTCTTCTACTACGGTACAACC ATCTTCAAAGCTGTTGGTATGTCCGATTCTTTTCAAACCTCTATAGTCTT GGGTATCGTTAACTTCGCTTCTACCTTTGTTGGTATTTGGGCCATTGAAA GAATGGGTAGAAGATCTTGTTTGTTGGTTGGTTCAGCTTGTATGTCTGTT TGCTTCTTGATCTACTCTATCTTGGGTTCAGTCAACTTGTACATCGATGG TTACGAAAACACTCCATCTAACACTAGAAAGCCAACTGGTAACGCCATG ATTTTCATTACCTGTTTGTTCATCTTTTTCTTCGCCTCTACTTGGGCTGGT GGTGTTTATTCTATAGTTTCTGAAACCTACCCATTGAGAATCAGATCTAA AGGTATGGCTGTTGCTACTGCTGCTAATTGGATGTGGGGTTTTTTGATCT CTTTCTTTACCCCATTCATCACCTCCGCTATTCATTTTTACTACGGTTTTG TTTTCACCGGTTGCTTGATCTTCTCATTCTTTTACGTATTCTTTTTCGTCC GTGAAACTAAGGGTTTGTCCTTGGAAGAAGTTGACGAATTATACGCTAC TGATTTGCCACCATGGAAAACTGCAGGTTGGACTCCACCATCAGCTGAA GATATGGCTCATACAACTGGTTTTGCTGAAGCTGCTAAGCCTACAAACA AACACGTTTGA

[0046] Expression of more than one xylose transporters can further improve xylose uptake. As such, the non-naturally occurring microbial organisms can have at least one exogenous nucleic acid, or at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or at least eleven nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least two exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least three exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least four exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least five exogenous nucleic acids each encoding a xylose transporter. In

some embodiments, the microbial organisms have at least six exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least seven exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least eight exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least nine exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least ten exogenous nucleic acids each encoding a xylose transporter. In some embodiments, the microbial organisms have at least eleven exogenous nucleic acids each encoding a xylose transporter.

[0047] The xylose transporters provided herein can be a *Metschnikowia* xylose transporter, including such as those from *H0 Metschnikowia sp.* having amino acid sequences as shown in sequence listing, as well as their variants that retain their transporter function. For example, provided herein is Xyt1p from *H0 Metschnikowia sp.* that has an amino acid sequence of SEQ ID NO:1, as well as variants thereof that retain the transporter function of Xyt1p. The transporter function of Xyt1p includes, but is not limited to, transport of xylose across cell wall and/or cell membrane, which can be determined, for example, by subjecting the variant to a transporter assay as described herin or otherwise known in the art. The xylose transporter function can be determined, for example, by expressing the transporter in a microbial organism and measuring the increase in xylose uptake by the microbial organism. In an exemplary assay, a non-xylose utilizing microbial organism expressing an exogenous transporter can be cultured in a xylose-contaning medium and the decrease of xylose in the culture medium can be measured by high performance liquid chromatography (HPLC) using Rezex RPM-monosaccharide Pb+2 column (Phenomenex), refractive index detector and water as a mobile phase at 0.6 ml/min. In another exemplary assay, starter cultures for wild type and transgenic microbial organisms expressing various transporters can be grown in YP base medium with controlled amounts of glucose and xylose (%; w/v). Uninoculated medium is used a reference for a given sampling time; the medium indicates 100% of the starting xylose or xylose at time 0 h. At 24 h intervals, samples at volumes of 300-1000 μL can be removed from the culture aseptically and filtered through a 0.2 μm syringe filter, physically separating medium and yeast. The medium can be transferred to glass vials and the xylose content can be examined by HPLC. The amount of xylose remaining in the sampled medium can be determined by comparison with a pre-defined calibration curve, and the remaining sample is normalized to the xylose content in the uninoculated medium, which

is counted as containing 100% of the xylose at the initiation of the culture. The non-naturally ocurring microbial organisms expressing an exogenous xylose transporter can consume xylose at a higher rate than their wild type counterparts, and the differences in the decrease rate of xylose in the culture medium between wild type and non-naturally ocurring microbial organisms expressing an exogenous xylose transporter can indicate the transporter function of the exogenous xylose transporter.

[0048] In some embodiments, provided herein are also isolated polypeptides that are variants of a *Metschnikowia* xylose transporter that retains its transporter function. Provided herein are also isolated nucleic acids that encode polypeptides that are variants to a *Metschnikowia* xylose transporter that retains its transporter function. In some embodiments, the variant of a *Metschnikowia* xylose transporter is ubiquitin-deficient. In some embodiments, the ubiquitin-deficient *Metschnikowia* xylose transporter has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient *Metschnikowia* xylose transporter has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient *Metschnikowia* xylose transporter has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient *Metschnikowia* xylose transporter has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient *Metschnikowia* xylose transporter has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, provided herein are non-naturally occurring microbial organisms having an exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter is a variant of a *Metschnikowia* xylose transporter that retains its transporter function. In some embodiments, the xylose transporter is a variant of the xylose transporters from the *H0 Metschnikowia sp.* as described herein that retains its transporter function. In some embodiments, the variant of a *H0 Metschnikowia sp.* xylose transporter is ubiquitin-deficient.

[0049] In some embodiments, provided herein are also isolated polypeptides that are variants of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species that retain the transporter

function. Provided herein are also isolated polypeptides that are variants of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from the *H0 Metschnikowia sp.* that retain the transporter function. In some embodiments, the variant is ubiquitin-deficient. In some embodiments, provided herein are also isolated nucleic acids that encode polypeptides that are variants of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species that retain the transporter function. In some embodiments, provided herein are also isolated nucleic acids that encode polypeptides that are variants of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from the *H0 Metschnikowia sp.* that retain the transporter function.

[0050] In some embodiments, provided herein are non-naturally occurring microbial organisms having an exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter is a variant of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species that retains the transporter function. In some embodiments, provided herein are non-naturally occurring microbial organisms having an exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter is a variant of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from *H0 Metschnikowia sp.* that retains the transporter function. In some embodiments, the variant is ubiquitin-deficient.

[0051] The xylose transporters described herein can have amino acid sequence of at least 30% identity, at least 35% identity, at least 40% identity, at least 45% identity, at least 50% identity, at least 55% identity, at least 60% identity, at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, or at least 99% identity, or is identical, to the amino acid sequences disclosed herein by SEQ ID NO, GenBank and/or GI number. In some embodiments, the xylose transporters described herein can have amino acid sequence of 35%, 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity, or be identical, to amino acids described herein by SEQ ID NO, GenBank and/or GI

number. In some embodiments, the xylose transporters described herein can have amino acid sequence of 35%, 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity, or be identical, to any one of SEQ ID NOs: 1-5 and 7-12.

5 **[0052]** In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 10 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* xylose transporter such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide 15 that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, 20 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* xylose transporter such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

[0053] In some embodiments, provided herein are non-naturally occurring microbial 25 organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 30 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* xylose transporter such as *Metschnikowia* Xyt1p,

Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p. In some embodiments, the exogenous nucleic acid encodes a *Metschnikowia* xylose transporter such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

5 **[0054]** Sequence identity (also known as homology or similarity) refers to sequence similarity between two nucleic acid molecules or between two polypeptides. Identity can be determined by comparing a position in each sequence, which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are identical at that position. A degree of identity between 10 sequences is a function of the number of matching or homologous positions shared by the sequences. The alignment of two sequences to determine their percent sequence identity can be done using software programs known in the art, such as, for example, those described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, MD (1999). Preferably, default parameters are used for the alignment. One alignment 15 program well known in the art that can be used is BLAST set to default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein 20 + SPupdate + PIR. Details of these programs can be found at the National Center for Biotechnology Information.

[0055] Variants of a specific xylose transporter can also include, for example, amino acid substitutions, deletions, fusions, or truncations when compared to the reference xylose transporter. Variants of the *Metschnikowia* xylose transporters described herein can also 25 contain conservatively amino acids substitution, meaning that one or more amino acid can be replaced by an amino acid that does not alter the secondary and/or tertiary stricture of the xylose transporter. Such substitutions can include the replacement of an amino acid, by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu, or Ala) for another, or substitutions between basic residues Lys and Arg, acidic 30 residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Phenotypically silent amino acid exchanges are described more fully in Bowie *et al.*, *Science* 247:1306-10 (1990). In addition, variants of

Metschnikowia xylose transporters include those having amino acid substitutions, deletions, or additions to the amino acid sequence outside functional regions of the protein so long as the substitution, deletion, or addition does not affect xylose transport function of the resulting polypeptide. In some embodiments, the variant is ubiquitin-deficient. Techniques for making these substitutions and deletions are well known in the art and include, for example, site-directed mutagenesis.

[0056] In some embodiments, provided herein are non-naturally occurring microbial organisms having an exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species and retains the transporter function. The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species and retains the transporter function. The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species and retains the transporter function. The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiment, the xylose transporter is a ubiquitin-deficient Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from a *Metschnikowia* species and retains the transporter function. The *Metschnikowia* species can be the *H0 Metschnikowia sp.*

[0057] The xylose transporters provided herein also include functional fragments of specific *Metschnikowia* xylose transporters that retain their transporter function. In some embodiments, provided herein is an isolated polypeptide that is a functional fragment of a specific *Metschnikowia* xylose transporter. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that is functional fragment of a specific *Metschnikowia* xylose transporter. In some embodiments, the xylose transporter can be fragments of a xylose transporter such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p

retains the transporter function. In some embodiments, the xylose transporter can be fragments of a xylose transporter such as Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p from the *H0 Metschnikowia sp.* retains the transporter function.

5 **[0058]** In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 10 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a function fragment of a *Metschnikowia* xylose transporter including such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p. In some embodiments, provided herein is an isolated nucleic acid 15 that encodes a polypeptide that has an amino acid sequence that is at least 30%, at at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 20 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a function fragment of a *Metschnikowia* xylose transporter including such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

[0059] In some embodiments, provided herein is an isolated polypeptide that has an 25 amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 30 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a function fragment of a xylose transporter of *H0 Metschnikowia sp.* including such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p,

Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a function fragment of a xylose transporter of *H0 Metschnikowia sp.* including such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

[0060] In some embodiments, provided herein are non-naturally occurring microbial organisms having an exogenous nucleic acid encoding a functional fragment of a *Metschnikowia* xylose transporter that retains its transporter function. In some embodiments, the non-naturally occurring microbial organisms provided herein have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a function fragment of a *Metschnikowia* xylose transporter including such as *Metschnikowia* Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p. In some embodiments, the non-naturally occurring microbial organisms provided herein have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a function fragment of a xylose transporter of *H0 Metschnikowia sp.* such as

Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, or Aps1p/Hgt19p.

[0061] In some embodiments, variants of *Metschnikowia* xylose transporters described herein include covalent modification or aggregative conjugation with other chemical moieties, such as glycosyl groups, polyethylene glycol (PEG) groups, lipids, phosphate, acetyl groups, and the like. In some embodiments, variants of the *Metschnikowia* xylose transporters described herein further include, for example, fusion proteins formed of xylose transporter polypeptide and another polypeptide. The added polypeptides for constructing the fusion protein include those that facilitate purification or oligomerization of xylose transporters, or those that enhance stability and/or transport capacity or transport rate of xylose transporters. In some embodiments, the added polypeptides gain enhanced transport capability when fused with the xylose transporters described herein.

[0062] The *Metschnikowia* xylose transporters described herein can be fused to heterologous polypeptides to facilitate purification. Many available heterologous peptides (peptide tags) allow selective binding of the fusion protein to a binding partner. Non-limiting examples of peptide tags include 6-His, thioredoxin, hemaglutinin, GST, and the OmpA signal sequence tag. A binding partner that recognizes and binds to the heterologous peptide tags can be any molecule or compound, including metal ions (for example, metal affinity columns), antibodies, antibody fragments, or any protein or peptide that selectively or specifically binds the heterologous peptide to permit purification of the fusion protein.

[0063] The *Metschnikowia* xylose transporters can also be modified to facilitate formation of oligomers. For example, the Xyt1p polypeptides can be fused to peptide moieties that promote oligomerization, such as leucine zippers and certain antibody fragment polypeptides, such as Fc polypeptides. Techniques for preparing these fusion proteins are known, and are described, for example, in WO 99/31241 and in Cosman *et al.*, *Immunity* 14:123-133 (2001). Fusion to an Fc polypeptide offers the additional benefit of facilitating purification by affinity chromatography over Protein A or Protein G columns. Fusion to a leucine-zipper (LZ), for example, a repetitive heptad repeat, often with four or five leucine residues interspersed with other amino acids, is described in Landschulz *et al.*, *Science* 240:1759-64 (1988).

[0064] The xylose transporters described herein can be provided in an isolated form, or in a substantially purified form. The polypeptides can be recovered and purified from recombinant cell cultures by known methods, including, for example, ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. In some embodiments, protein chromatography is employed for purification.

[0065] The *Metschnikowia* xylose transporters described herein can be recombinantly expressed by suitable hosts. When heterologous expression of the *Metschnikowia* xylose transporters is desired, the coding sequences of specific *Metschnikowia* xylose transporters can be modified in accordance with the codon usage of the host. The standard genetic code is well known in the art, as reviewed in, for example, Osawa *et al.*, *Microbiol Rev.* 56(1):229-64 (1992). Yeast species, including but not limited to *Saccharomyces cerevisiae*, *Candida azyma*, *Candida diversa*, *Candida magnoliae*, *Candida rugopelliculosa*, *Yarrowia lipolytica*, and *Zygoascus hellenicus*, use the standard code. Certain yeast species use alternative codes. For example, "CUG," standard codon for "Leu," encodes "Ser" in species such as *Candida albicans, Candida cylindracea, Candida melibiosica, Candida parapsilosis, Candida rugose, Pichia stipitis, and Metschnikowia* species. The codon table for the *H0 Metschnikowia sp.* is provided below. The DNA codon CTG in a foreign gene from a non "CUG" clade species need to be changed to TTG, CTT, CTC, TTA, or CTA for a functional expression of a protein in the *Metschnikowia* species. Other codon optimization can result in increase of protein expression of a foreign gene in the *Metschnikowia* species. Codon optimization can result in increase protein expression of a foreign gene in the host. Methods of Codon optimization are well known in the art (*e.g.* Chung *et al.*, *BMC Syst Biol.* 6:134 (2012); Chin *et al.*, *Bioinformatics* 30(15):2210-12 (2014)), and various tools are available (*e.g.* DNA2.0 at https://www.dna20.com/services/genegps; and OPTIMIZER at http://genomes.urv.es/OPTIMIZER).

[0066] Table: Codons for *H0 Metschnikowia sp.*

Amino Acid	SLC	DNA codons					
Isoleucine	I	ATT	ATC	ATA			
Leucine	L	CTT	CTC	CTA		TTA	TTG
Valine	V	GTT	GTC	GTA	GTG		
Phenylalanine	F	TTT	TTC				

Methionine	M	ATG
Cysteine	C	TGT TGC
Alanine	A	GCT GCC GCA GCG
Glycine	G	GGT GGC GGA GGG
Proline	P	CCT CCC CCA CCG
Threonine	T	ACT ACC ACA ACG
Serine	S	TCT TCC TCA TCG AGT AGC CTG
Tyrosine	Y	TAT TAC
Tryptophan	W	TGG
Glutamine	Q	CAA CAG
Asparagine	N	AAT AAC
Histidine	H	CAT CAC
Glutamic acid	E	GAA GAG
Aspartic acid	D	GAT GAC
Lysine	K	AAA AAG
Arginine	R	CGT CGC CGA CGG AGA AGG
Stop codons	Stop	TAA TAG TGA

[0067] Furthermore, the hosts can simultaneously produce other transporters such that multiple transporters are expressed in the same cell, wherein the different transporters can form oligomers to transport the same sugar. Alternatively, the different transporters can
5 function independently to transport different sugars.

[0068] Variants of *Metschnikowia* xylose transporters can be generated by conventional methods known in the art, such as by introducing mutations at particular locations by oligonucleotide-directed site-directed mutagenesis. Site-directed-mutagenesis is considered an informational approach to protein engineering and can rely on high-resolution
10 crystallographic structures of target proteins for specific amino acid changes (Van Den Burg *et al.*, *PNAS* 95:2056-60 (1998)). Computational methods for identifying site-specific changes for a variety of protein engineering objectives are also known in the art (Hellinga, *Nature Structural Biology* 5:525-27 (1998)).

[0069] Other techniques known in the art include, but are not limited to, non-
15 informational mutagenesis techniques (referred to generically as "directed evolution"). Directed evolution, in conjunction with high-throughput screening, allows testing of statistically meaningful variations in protein conformation (Arnold, 1998). Directed evolution technology can include diversification methods similar to that described by Crameri *et al.*, *Nature* 391:288-91 (1998), site-saturation mutagenesis, staggered extension process (StEP)

(Zhao *et al.*, *Nature Biotechnology* 16:258-61 (1998)), and DNA synthesis/reassembly (U.S. Pat. No. 5,965,408).

[0070] As disclosed herein, a nucleic acid encoding xylose transporter can be introduced into a host organism. In some cases, it can also be desirable to modify an activity of a biosynthesis pathway enzyme or protein to increase production of a desired product. For example, known mutations that increase the activity of a protein or enzyme can be introduced into an encoding nucleic acid molecule. Additionally, optimization methods can be applied to increase the activity of an enzyme or protein and/or decrease an inhibitory activity, for example, decrease the activity of a negative regulator.

[0071] One such optimization method is directed evolution. Directed evolution is a powerful approach that involves the introduction of mutations targeted to a specific gene in order to improve and/or alter the properties of an enzyme. Improved and/or altered enzymes can be identified through the development and implementation of sensitive high-throughput screening assays that allow the automated screening of many enzyme variants (for example, $>10^4$). Iterative rounds of mutagenesis and screening typically are performed to afford an enzyme with optimized properties. Computational algorithms that can help to identify areas of the gene for mutagenesis also have been developed and can significantly reduce the number of enzyme variants that need to be generated and screened. Numerous directed evolution technologies have been developed (for reviews, see Hibbert et al., *Biomol.Eng* 22:11-19 (2005); Huisman and Lalonde, *In* Biocatalysis in the pharmaceutical and biotechnology industries pgs. 717-742 (2007), Patel (ed.), CRC Press; Otten and Quax. *Biomol.Eng* 22:1-9 (2005).; and Sen et al., *Appl Biochem.Biotechnol* 143:212-223 (2007)) to be effective at creating diverse variant libraries, and these methods have been successfully applied to the improvement of a wide range of properties across many enzyme classes. Enzyme characteristics that have been improved and/or altered by directed evolution technologies include, for example: selectivity/specificity, for conversion of non-natural substrates; temperature stability, for robust high temperature processing; pH stability, for bioprocessing under lower or higher pH conditions; substrate or product tolerance, so that high product titers can be achieved; binding (K_m), including broadening substrate binding to include non-natural substrates; inhibition (K_i), to remove inhibition by products, substrates, or key intermediates; activity (kcat), to increases enzymatic reaction rates to achieve desired flux; expression levels, to increase protein yields and overall pathway flux; oxygen stability,

for operation of air sensitive enzymes under aerobic conditions; and anaerobic activity, for operation of an aerobic enzyme in the absence of oxygen.

[0072] A number of exemplary methods have been developed for the mutagenesis and diversification of genes to target desired properties of specific enzymes. Such methods are well known to those skilled in the art. Any of these can be used to alter and/or optimize the activity of a xylose transporter or a biosynthesis pathway enzyme or protein. Such methods include, but are not limited to EpPCR, which introduces random point mutations by reducing the fidelity of DNA polymerase in PCR reactions (Pritchard et al., *J Theor.Biol.* 234:497-509 (2005)); Error-prone Rolling Circle Amplification (epRCA), which is similar to epPCR except a whole circular plasmid is used as the template and random 6-mers with exonuclease resistant thiophosphate linkages on the last 2 nucleotides are used to amplify the plasmid followed by transformation into cells in which the plasmid is re-circularized at tandem repeats (Fujii et al., *Nucleic Acids Res.* 32:e145 (2004); and Fujii et al., *Nat. Protoc.* 1:2493-2497 (2006)); DNA or Family Shuffling, which typically involves digestion of two or more variant genes with nucleases such as Dnase I or EndoV to generate a pool of random fragments that are reassembled by cycles of annealing and extension in the presence of DNA polymerase to create a library of chimeric genes (Stemmer, *Proc Natl Acad Sci USA* 91:10747-10751 (1994); and Stemmer, *Nature* 370:389-391 (1994)); Staggered Extension (StEP), which entails template priming followed by repeated cycles of 2 step PCR with denaturation and very short duration of annealing/extension (as short as 5 sec) (Zhao et al., *Nat. Biotechnol.* 16:258-261 (1998)); Random Priming Recombination (RPR), in which random sequence primers are used to generate many short DNA fragments complementary to different segments of the template (Shao et al., *Nucleic Acids Res* 26:681-683 (1998)).

[0073] Additional methods include Heteroduplex Recombination, in which linearized plasmid DNA is used to form heteroduplexes that are repaired by mismatch repair (Volkov et al, *Nucleic Acids Res.* 27:e18 (1999); and Volkov et al., *Methods Enzymol.* 328:456-463 (2000)); Random Chimeragenesis on Transient Templates (RACHITT), which employs Dnase I fragmentation and size fractionation of single stranded DNA (ssDNA) (Coco et al., *Nat. Biotechnol.* 19:354-359 (2001)); Recombined Extension on Truncated templates (RETT), which entails template switching of unidirectionally growing strands from primers in the presence of unidirectional ssDNA fragments used as a pool of templates (Lee et al., *J. Molec. Catalysis* 26:119-129 (2003)); Degenerate Oligonucleotide Gene Shuffling (DOGS),

in which degenerate primers are used to control recombination between molecules; (Bergquist and Gibbs, *Methods Mol.Biol* 352:191-204 (2007); Bergquist et al., *Biomol.Eng* 22:63-72 (2005); Gibbs et al., *Gene* 271:13-20 (2001)); Incremental Truncation for the Creation of Hybrid Enzymes (ITCHY), which creates a combinatorial library with 1 base pair deletions of a gene or gene fragment of interest (Ostermeier et al., *Proc. Natl. Acad. Sci. USA* 96:3562-3567 (1999); and Ostermeier et al., *Nat. Biotechnol.* 17:1205-1209 (1999)); Thio-Incremental Truncation for the Creation of Hybrid Enzymes (THIO-ITCHY), which is similar to ITCHY except that phosphothioate dNTPs are used to generate truncations (Lutz et al., *Nucleic Acids Res* 29:E16 (2001)); SCRATCHY, which combines two methods for recombining genes, ITCHY and DNA shuffling (Lutz et al., *Proc. Natl. Acad. Sci. USA* 98:11248-11253 (2001)); Random Drift Mutagenesis (RNDM), in which mutations made via epPCR are followed by screening/selection for those retaining usable activity (Bergquist et al., *Biomol. Eng.* 22:63-72 (2005)); Sequence Saturation Mutagenesis (SeSaM), a random mutagenesis method that generates a pool of random length fragments using random incorporation of a phosphothioate nucleotide and cleavage, which is used as a template to extend in the presence of "universal" bases such as inosine, and replication of an inosine-containing complement gives random base incorporation and, consequently, mutagenesis (Wong et al., *Biotechnol. J.* 3:74-82 (2008); Wong et al., *Nucleic Acids Res.* 32:e26 (2004); and Wong et al., *Anal. Biochem.* 341:187-189 (2005)); Synthetic Shuffling, which uses overlapping oligonucleotides designed to encode "all genetic diversity in targets" and allows a very high diversity for the shuffled progeny (Ness et al., *Nat. Biotechnol.* 20:1251-1255 (2002)); Nucleotide Exchange and Excision Technology NexT, which exploits a combination of dUTP incorporation followed by treatment with uracil DNA glycosylase and then piperidine to perform endpoint DNA fragmentation (Muller et al., *Nucleic Acids Res.* 33:e117 (2005)).

[0074] Further methods include Sequence Homology-Independent Protein Recombination (SHIPREC), in which a linker is used to facilitate fusion between two distantly related or unrelated genes, and a range of chimeras is generated between the two genes, resulting in libraries of single-crossover hybrids (Sieber et al., *Nat. Biotechnol.* 19:456-460 (2001)); Gene Site Saturation Mutagenesis™ (GSSM™), in which the starting materials include a supercoiled double stranded DNA (dsDNA) plasmid containing an insert and two primers which are degenerate at the desired site of mutations (Kretz et al., *Methods Enzymol.* 388:3-11 (2004)); Combinatorial Cassette Mutagenesis (CCM), which involves the

use of short oligonucleotide cassettes to replace limited regions with a large number of possible amino acid sequence alterations (Reidhaar-Olson et al. *Methods Enzymol.* 208:564-586 (1991); and Reidhaar-Olson et al. *Science* 241:53-57 (1988)); Combinatorial Multiple Cassette Mutagenesis (CMCM), which is essentially similar to CCM and uses epPCR at high
5 mutation rate to identify hot spots and hot regions and then extension by CMCM to cover a defined region of protein sequence space (Reetz et al., *Angew. Chem. Int. Ed Engl.* 40:3589-3591 (2001)); the Mutator Strains technique, in which conditional *ts* mutator plasmids, utilizing the *mutD5* gene, which encodes a mutant subunit of DNA polymerase III, to allow increases of 20 to 4000-X in random and natural mutation frequency during selection and
10 block accumulation of deleterious mutations when selection is not required (Selifonova et al., *Appl. Environ. Microbiol.* 67:3645-3649 (2001)); Low et al., *J. Mol. Biol.* 260:359-3680 (1996)).

[0075] Additional exemplary methods include Look-Through Mutagenesis (LTM), which is a multidimensional mutagenesis method that assesses and optimizes combinatorial
15 mutations of selected amino acids (Rajpal et al., *Proc. Natl. Acad. Sci. USA* 102:8466-8471 (2005)); Gene Reassembly, which is a DNA shuffling method that can be applied to multiple genes at one time or to create a large library of chimeras (multiple mutations) of a single gene (Tunable GeneReassembly™ (TGR™) Technology supplied by Verenium Corporation), *in Silico* Protein Design Automation (PDA), which is an optimization algorithm that anchors the
20 structurally defined protein backbone possessing a particular fold, and searches sequence space for amino acid substitutions that can stabilize the fold and overall protein energetics, and generally works most effectively on proteins with known three-dimensional structures (Hayes et al., *Proc. Natl. Acad. Sci. USA* 99:15926-15931 (2002)); and Iterative Saturation Mutagenesis (ISM), which involves using knowledge of structure/function to choose a likely
25 site for enzyme improvement, performing saturation mutagenesis at chosen site using a mutagenesis method such as Stratagene QuikChange (Stratagene; San Diego CA), screening/selecting for desired properties, and, using improved clone(s), starting over at another site and continue repeating until a desired activity is achieved (Reetz et al., *Nat. Protoc.* 2:891-903 (2007); and Reetz et al., *Angew. Chem. Int. Ed Engl.* 45:7745-7751
30 (2006)).

[0076] Any of the aforementioned methods for mutagenesis can be used alone or in any combination. Additionally, any one or combination of the directed evolution methods can be

used in conjunction with adaptive evolution techniques, as described herein or otherwise known in the art.

[0077] Provided herein are also isolated nucleic acids encoding the *Metschnikowia* xylose transporters described herein. Nucleic acids provided herein include those having the nucleic acid sequence provided in the sequence listing; those that hybridize to the nucleic acid sequences provided in the sequence listing, under high stringency hybridization conditions (for example, 42°, 2.5 hr., 6×SCC, 0.1% SDS); and those having substantial nucleic acid sequence identity with the nucleic acid sequence provided in the sequence listing. The nucleic acids provided herein also encompass equivalent substitutions of codons that can be translated to produce the same amino acid sequences. Provided herein are also vectors including the nucleic acids described herein. The vector can be an expression vector suitable for expression in a host microbial organism. The vector can be a 2 μ vector. The vector can be an ARS vector.

[0078] The nucleic acids provided herein include those encoding xylose transporters having an amino acid sequence as described herein, as well as their variants that retain transporter activity. The nucleic acids provided herein can be cDNA, chemically synthesized DNA, DNA amplified by PCR, RNA, or combinations thereof. Due to the degeneracy of the genetic code, two DNA sequences can differ and yet encode identical amino acid sequences.

[0079] Provided herein are also useful fragments of nucleic acids encoding the *Metschnikowia* xylose transporters described herein, include probes and primers. Such probes and primers can be used, for example, in PCR methods to amplify or detect the presence of nucleic acids encoding the *Metschnikowia* xylose transporters in vitro, as well as in Southern and Northern blots for analysis. Cells expressing the *Metschnikowia* xylose transporters can also be identified by the use of such probes. Methods for the production and use of such primers and probes are known.

[0080] Provided herein are also fragments of nucleic acids encoding the *Metschnikowia* xylose transporters that are antisense or sense oligonucleotides having a single-stranded nucleic acid capable of binding to a target mRNA or DNA sequence of a *Metschnikowia* xylose transporter.

[0081] A nucleic acid encoding a xylose transporter described herein can include nucleic acids that hybridize to a nucleic acid disclosed herein by SEQ ID NO, GenBank and/or GI number or a nucleic acid molecule that hybridizes to a nucleic acid molecule that encodes an amino acid sequence disclosed herein by SEQ ID NO, GenBank and/or GI number.
5 Hybridization conditions can include highly stringent, moderately stringent, or low stringency hybridization conditions that are well known to one of skill in the art such as those described herein.

[0082] Stringent hybridization refers to conditions under which hybridized polynucleotides are stable. As known to those of skill in the art, the stability of hybridized
10 polynucleotides is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of hybridized polynucleotides is a function of the salt concentration, for example, the sodium ion concentration and temperature. A hybridization reaction can be performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions. Highly stringent
15 hybridization includes conditions that permit hybridization of only those nucleic acid sequences that form stable hybridized polynucleotides in 0.018M NaCl at 65°C, for example, if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein. High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at
20 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C. Hybridization conditions other than highly stringent hybridization conditions can also be used to describe the nucleic acid sequences disclosed herein. For example, the phrase moderately stringent hybridization refers to conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 42°C. The
25 phrase low stringency hybridization refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 22°C, followed by washing in 1X SSPE, 0.2% SDS, at 37°C. Denhart's solution contains 1% Ficoll, 1% polyvinylpyrolidone, and 1% bovine serum albumin (BSA). 20X SSPE (sodium chloride, sodium phosphate, ethylene diamide tetraacetic acid (EDTA)) contains 3M sodium chloride, 0.2M sodium
30 phosphate, and 0.025 M (EDTA). Other suitable low, moderate and high stringency hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Third

Ed., Cold Spring Harbor Laboratory, New York (2001); and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, MD (1999).

[0083] Nucleic acids encoding a xylose transporter provided herein include those having a certain percent sequence identity to a nucleic acid disclosed herein by SEQ ID NO, GenBank and/or GI number. For example, the nucleic acids encoding a xylose transporter can have at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or be identical, to a nucleic acid described herein by SEQ ID NO, GenBank and/or GI number. In some embodiments, the nucleic acid molecule can have 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity, or be identical, to a sequence selected from SEQ ID NOs: 10-16 and 19-27.

[0084] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Xyt1p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Xyt1p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic

acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Xyt1p. In some embodiments, the xylose transporter can be a *Metschnikowia* Xyt1p. In some embodiments, the xylose transporter can be a variant of a *Metschnikowia* Xyt1p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Xyt1p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Xyt1p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Xyt1p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Xyt1p from a *Metschnikowia* species.

[0085] In some embodiment, the xylose transporter is an ubiquitin-deficient Xyt1p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid mutations at or near at least two lysine residues that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid mutations at or near at least three lysine residues that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Xyt1p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, the lysine residues that can be ubiquitinated include K6, K517 and K539 of Xyt1p. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at one of K6, K517 and K539. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitution at K6. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitution at K517. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitution at

K539. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at two of K6, K517 and K539. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at K517 and K539. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at K539 and K6. In some embodiments, the ubiquitin-deficient
5 Xyt1p has amino acid substitutions at K6 and K517. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at three of K6, K517 and K539.

[0086] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least
10 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Xyt1p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide
15 that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%,
20 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Xyt1p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least
25 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Xyt1p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Xyt1p of *H0*
30 *Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Xyt1p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiment, the xylose transporter is a ubiquitin-deficient Xyt1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is a functional fragment of Xyt1p of *H0 Metschnikowia sp.* In some

embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Xyt1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Xyt1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Xyt1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 1. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 1. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 13. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 13. In some embodiments, the ubiquitin-deficient Xyt1p has the amino acid sequence of SEQ ID NO: 48. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 53. The nucleic acid encoding Xyt1p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Xyt1p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Xyt1p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Xyt1p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*. For example, in some embodiments, the nucleic acid encoding Xyt1p of *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*. The nucleic acid can have the sequence of SEQ ID NO: 21.

[0087] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf1p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%,

at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf1p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf1p. In some embodiments, the xylose transporter is a *Metschnikowia* Gxf1p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Gxf1pthat retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Gxf1p. In some embodiments, the nucleic acid encodes a xylose transporter having 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxf1p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxf1p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxf1p from a *Metschnikowia* species.

[0088] In some embodiment, the xylose transporter is a ubiquitin-deficient Gxf1p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Gxf1p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not

accessible to the ubiquitination machinery. In some embodiments, the lysine residues that can be ubiquitinated include K9, K24, and K538 of Gxf1p. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at one of K9, K24, and K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitution at K9. In some
5 embodiments, the ubiquitin-deficient Gxf1p has amino acid substitution at K24. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitution at K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at two of K9, K24, and K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at K9 and K24. . In some embodiments, the ubiquitin-deficient Gxf1p has amino acid
10 substitutions at K538 and K9. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at K24 and K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at three of K9, K24, and K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at K9, K24, K538. In some embodiments, the ubiquitin-deficient Gxf1p has amino acid substitutions at K9, K24, and
15 K538.

[0089] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least
20 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Gxf1pof *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that
25 encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at
30 least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Gxf1pof *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at

least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at
5 least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Gxf1p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Gxf1p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Gxf1p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiment, the xylose transporter is a ubiquitin-deficient Gxf1p from *H0 Metschnikowia*
10 *sp.* In some embodiments, the xylose transporter is a functional fragment of Gxf1p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxf1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxf1p from
15 *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxf1p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 2. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 2. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 14. In some embodiments,
20 the sequence of the nucleic acid is SEQ ID NO: 14. In some embodiments, the ubiquitin-deficient Gxf1p has the amino acid sequence of SEQ ID NO: 47. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 52. In some embodiments, the nucleic acid encodes a functional fragment of Gxf1p of *H0 Metschnikowia sp.* For example, the fragment of Gxf1p can be a variant of Gxf1p that has a shorter N-terminus, and referred to as ΔGxf1p.
25 In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 3. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 15. The nucleic acid encoding Gxf1p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxf1p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host
30 strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxf1p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as

E. coli. In some embodiments, the nucleic acid encoding Gxf1p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*.

[0090] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf2p/Gal2p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf2p/Gal2p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxf2p/Gal2p. In some embodiments, the xylose transporter is a *Metschnikowia* Gxf2p/Gal2p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Gxf2p/Gal2p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Gxf2p/Gal2p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from a

Metschnikowia species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from a *Metschnikowia* species.

[0091] In some embodiment, the xylose transporter is a ubiquitin-deficient Gxf2p/Gal2p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutations at or near at least four lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Gxf2p/Gal2p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, the lysine residues that can be ubiquitinated include K23, K26, K35, K542 and K546 of Gxf2p/Gal2p. In some embodiments, the ubiquitin-deficient Xyt1p has amino acid substitutions at one of K23, K26, K35, K542 and K546. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitution at K23. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitution at K26. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitution at K35. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitution at K542. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitution at K546. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitutions at two of K23, K26, K35, K542 and K546. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitutions at three of K23, K26, K35, K542 and K546. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitutions at four of K23, K26, K35, K542 and K546. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has amino acid substitutions at K23, K26, K35, K542 and K546.

[0092] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that

is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Gxf2p/Gal2p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Gxf2p/Gal2p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Gxf2p/Gal2p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Gxf2p/Gal2p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Gxf2p/Gal2p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiment, the xylose transporter is a ubiquitin-deficient Gxf2p/Gal2p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is a functional fragment of Gxf2p/Gal2p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxf2p/Gal2p from *H0 Metschnikowia sp.* In some embodiments, the xylose

transporter has the amino acid sequence of SEQ ID NO: 4. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 4. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 16. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 16. In some embodiments, the ubiquitin-deficient Gxf2p/Gal2p has the amino acid sequence of SEQ ID NO: 46. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 51. The nucleic acid encoding Gxf2p/Gal2p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxf2p/Gal2p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxf2p/Gal2p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Gxf2p/Gal2p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*.

[0093] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxs1p/Hgt12p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxs1p/Hgt12p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least

72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Gxs1p/Hgt12p. In some embodiments, the xylose transporter is a *Metschnikowia* Gxs1p/Hgt12p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Gxs1p/Hgt12p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Gxs1p/Hgt12p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from a *Metschnikowia* species.

[0094] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Gxs1p/Hgt12p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Gxs1p/Hgt12p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at

least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Gxs1p/Hgt12p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Gxs1p/Hgt12p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Gxs1p/Hgt12p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiments, the xylose transporter is a functional fragment of Gxs1p/Hgt12p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Gxs1p/Hgt12p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 7. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 7. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 19. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 19. In some embodiments, the nucleic acid encodes a functional fragment of Gxs1p/Hgt12p of *H0 Metschnikowia sp.* For example, the fragment of Gxs1p/Hgt12p can be a variant of Gxs1p/Hgt12p that has a shorter N-terminus, and referred to as ΔGxs1p/ΔHgt12p. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 5. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 17. The nucleic acid encoding Gxs1p/Hgt12p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxs1p/Hgt12p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Gxs1p/Hgt12p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Gxs1p/Hgt12p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*.

[0095] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt5p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt5p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt5p. In some embodiments, the xylose transporter is a *Metschnikowia* Hxt5p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Hxt5p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Hxt5p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Hxt5p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Hxt5p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Hxt5p from a *Metschnikowia* species.

[0096] In some embodiment, the xylose transporter is a ubiquitin-deficient Hxt5p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Hxt5p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, the lysine residues that can be ubiquitinated include K7, K10, K29, K43 and K58 of Hxt5p. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at one of K7, K10, K29, K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitution at K7. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitution at K10. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitution at K29. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitution at K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitution at K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at two of K7, K10, K29, K43, and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7 and K10. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7 and K29. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10 and K29. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10 and K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K29 and K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K43 and K7. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K29 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at three of K7, K10, K29, K43 and K58. In some embodiments, the ubiquitin-

deficient Hxt5p has amino acid substitutions at K7, K10, and K29. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, and K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10, K29 and K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10, K29 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K29, K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at four of K7, K10, K29, K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, K29 and K43. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, K29 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K29, K43 and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K10, K29, K43, and K58. In some embodiments, the ubiquitin-deficient Hxt5p has amino acid substitutions at K7, K10, K29, K43 and K58.

[0097] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Hxt5p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Hxt5p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid

encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at 5 least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Hxt5p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Hxt5p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Hxt5p of *H0 Metschnikowia sp.* that retains its transporter function. In some 10 embodiment, the xylose transporter is a ubiquitin-deficient Hxt5p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is a functional fragment of Hxt5p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Hxt5p from *H0 Metschnikowia sp.* In some embodiments, the 15 xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Hxt5p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Hxt5p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 8. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 8. In some 20 embodiments, the nucleic acid has the sequence of SEQ ID NO: 20. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 20. In some embodiments, the ubiquitin-deficient Hxt5p has the amino acid sequence of SEQ ID NO: 45. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 50. The nucleic acid encoding Hxt5p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some 25 embodiments, the nucleic acid encoding *Metschnikowia* Hxt5p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Hxt5p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some 30 embodiments, the nucleic acid encoding Hxt5p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*.

[0098] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least

50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt2.6p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt2.6p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Hxt2.6p. In some embodiments, the xylose transporter is a *Metschnikowia* Hxt2.6p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Hxt2.6p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Hxt2.6p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Hxt2.6p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Hxt2.6p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Hxt2.6p from a *Metschnikowia* species. In some embodiment, the xylose transporter is a ubiquitin-deficient Hxt2.6p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Hxt2.6p has amino acid mutation at or near at least one lysine residue that can be

ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt2.6p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt2.6p has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Hxt2.6p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Hxt2.6p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery.

[0099] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Hxt2.6p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Hxt2.6p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Hxt2.6p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter

is Hxt2.6p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Hxt2.6p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiments, the xylose transporter is a functional fragment of Hxt2.6p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Hxt2.6p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Hxt2.6p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Hxt2.6p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 10. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 10. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 22. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 22. The Hxt2.6p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Hxt2.6p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Hxt2.6p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Hxt2.6p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*. For example, in some embodiments, the nucleic acid encodes Hxt2.6p of *H0 Metschnikowia sp.* that is codon optimized for expression in *Saccharomyces cerevisiae*. The nucleic acid can have the sequence of SEQ ID NO: 23.

[00100] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Qup2p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%,

at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, 5 at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Qup2p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 10 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a 15 *Metschnikowia* Qup2p. In some embodiments, the xylose transporter is a *Metschnikowia* Qup2p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Qup2p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Qup2p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid 20 substitutions, deletions or insertions of Qup2p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Qup2p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Qup2p from a *Metschnikowia* species. In some embodiment, the xylose transporter is a ubiquitin-deficient 25 Qup2p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Qup2p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Qup2p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Qup2p has amino acid mutations at or near at least three lysine residue that can be 30 ubiquitinated. In some embodiments, the ubiquitin-deficient Qup2p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient

Qup2p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery.

[00101] The *Metschnikowia* species can be the *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that
5 is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least
10 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Qup2p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at
15 least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Qup2p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid
20 encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at
25 least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to Qup2p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be Qup2p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Qup2p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiments, the xylose transporter can be a functional fragment of Qup2p of *H0*
30 *Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Qup2p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Qup2p from

H0 Metschnikowia sp. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Qup2p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 11. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 11. In
5 some embodiments, the nucleic acid has the sequence of SEQ ID NO: 24. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 24. The Qup2p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Qup2p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described
10 herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Qup2p is codon optimized for expression in a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Qup2p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*. For example, in some embodiments,
15 the nucleic acid encodes Qup2p of *H0 Metschnikowia sp.* that is codon optimized for expression in *Saccharomyces cerevisiae*. The nucleic acid can have the sequence of SEQ ID NO: 25.

[00102] In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least
20 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to
25 a *Metschnikowia* Aps1p/Hgt19p. In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%,
30 at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Aps1p/Hgt19p. In some embodiments, provided herein are non-naturally occurring microbial organisms having at

least one exogenous nucleic acid encoding a xylose transporter, wherein the xylose transporter has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a *Metschnikowia* Aps1p/Hgt19p. In some embodiments, the xylose transporter is a *Metschnikowia* Aps1p/Hgt19p. In some embodiments, the xylose transporter is a variant of a *Metschnikowia* Aps1p/Hgt19p that retains its transporter function. The xylose transporter can be a functional fragment of a *Metschnikowia* Aps1p/Hgt19p. In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from a *Metschnikowia* species. In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from a *Metschnikowia* species.

[00103] In some embodiment, the xylose transporter is a ubiquitin-deficient Aps1p/Hgt19p from a *Metschnikowia* species. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid mutation at or near at least one lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid mutations at or near at least two lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid mutations at or near at least three lysine residue that can be ubiquitinated. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid mutations at or near all lysine residue that can be ubiquitinated. In some embodiment, the amino acid mutation is substitution of the lysine residue. In some embodiment, the amino acid mutation is deletion of the lysine residue. In some embodiment, the ubiquitin-deficient Aps1p/Hgt19p has amino acid mutation near the lysine residue that can be ubiquitinated such that the lysine residue is not accessible to the ubiquitination machinery. In some embodiments, the lysine residues that can be ubiquitinated include K4, K20, K30 and K93 of Aps1p/Hgt19p. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at one of K4, K20, K30 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid

substitution at K4. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitution at K20. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitution at K30. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitution at K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at two of K4, K20, K30 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K4 and K20. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K20 and K30. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K30 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K93 and K4. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K4 and K30. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K20 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at three of K4, K20, K30 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K4, K20, and K30. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K20, K30 and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K30, K93 and K4. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K4, K20, and K93. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has amino acid substitutions at K4, K20, K30 and K93.

[00104] The *Metschnikowia* species can be the *Metschnikowia H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Aps1p/Hgt19p of *H0 Metschnikowia sp.* In some embodiments, provided herein is an isolated nucleic acid that encodes a polypeptide that has an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at

least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a Aps1p/Hgt19p of *H0 Metschnikowia sp.* In some embodiments, the non-naturally occurring microbial organisms have at least one exogenous nucleic acid encoding a xylose transporter that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Aps1p/Hgt19p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is Aps1p/Hgt19p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can be a variant of Aps1p/Hgt19p of *H0 Metschnikowia sp.* that retains its transporter function. In some embodiment, the xylose transporter is a ubiquitin-deficient Aps1p/Hgt19p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter is a functional fragment of Aps1p/Hgt19p of *H0 Metschnikowia sp.* In some embodiments, the xylose transporter can have 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5, amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 10 amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has 1 to 5 amino acid substitutions, deletions or insertions of Aps1p/Hgt19p from *H0 Metschnikowia sp.* In some embodiments, the xylose transporter has the amino acid sequence of SEQ ID NO: 12. In some embodiments, the amino acid sequence of the xylose transporter is SEQ ID NO: 12. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 26. In some embodiments, the sequence of the nucleic acid is SEQ ID NO: 26. In some embodiments, the ubiquitin-deficient Aps1p/Hgt19p has the amino acid sequence of SEQ ID NO: 44. In some embodiments, the nucleic acid has the sequence of SEQ ID NO: 49. The Aps1p/Hgt19p from a *Metschnikowia* species can be codon optimized for heterologous expression. In some embodiments, the nucleic acid encoding *Metschnikowia* Aps1p/Hgt19p is codon optimized for expression in a yeast host strain. The yeast host strain can be any yeast host strain described herein, such as *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid encoding *Metschnikowia* Aps1p/Hgt19p is codon optimized for expression in

a bacterial host strain. The bacterial host strain can be any bacterial host strain described herein, such as *E. coli*. In some embodiments, the nucleic acid encoding Aps1p/Hgt19p from *H0 Metschnikowia sp.* is codon optimized for expression in *Saccharomyces cerevisiae*. For example, in some embodiments, the nucleic acid encodes Aps1p/Hgt19p of *H0*
5 *Metschnikowia sp.* that is codon optimized for expression in *Saccharomyces cerevisiae*. The nucleic acid can have the sequence of SEQ ID NO: 27.

[00105] As provided above, the non-naturally occurring microbial organisms can have at least one exogenous nucleic acid, or at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine nucleic acids encoding a combination of
10 xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express two xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express three xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express four xylose transporters described herein. In some embodiments, the non-
15 naturally occurring microbial organisms express five xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express six xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express seven xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express eight xylose transporters described
20 herein. In some embodiments, the non-naturally occurring microbial organisms express nine xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express ten xylose transporters described herein. In some embodiments, the non-naturally occurring microbial organisms express eleven xylose transporters described herein. In some embodiments, the combination of xylose transporters include two, three,
25 four, five, six, seven, eight, nine, or ten xylose transporters of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, and Aps1p/Hgt19p from a *Metschnikowia* species as well as variants thereof. In some embodiments, the combination of xylose transporters include two, three, four, five, six, seven, eight, nine, or ten xylose transporters of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p,
30 ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, and Aps1p/Hgt19p from *H0 Metschnikowia sp.* as well as variants thereof.

[00106] The xylose transporter provided herein can be isolated by a variety of methods well-known in the art, for example, recombinant expression systems, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., *Guide to Protein Purification: Methods in Enzymology*, Vol. 182 (Academic Press, (1990)). Alternatively, the isolated xylose transporter provided herein can be obtained using well-known recombinant methods (see, for example, Sambrook et al., supra, 1989; Ausubel et al., supra, 1999). The methods and conditions for biochemical purification of the isolated xylose transporter provided herein can be chosen by those skilled in the art, and purification monitored, for example, by a functional assay.

[00107] One non-limiting example of a method for preparing the xylose transporter is to express nucleic acids encoding the xylose transporter in a suitable host cell, such as a bacterial cell, a yeast cell, or other suitable cell, using methods well known in the art, and recovering the expressed xylose transporter, again using well-known purification methods, as described herein. The xylose transporter provided herein can be isolated directly from cells that have been transformed with expression vectors as described herein. Recombinantly expressed xylose transporters can also be expressed as fusion proteins with appropriate affinity tags, such as glutathione S transferase (GST), poly His, streptavidin, and the like, and affinity purified, if desired. The polypeptide of the xylose transporters described herein can retain the affinity tag, if desired, or optionally the affinity tag can be removed from the polypeptide using well known methods to remove an affinity tag, for example, using appropriate enzymatic or chemical cleavage. Thus, provided herein are polypeptide of xylose transporters without or optionally with an affinity tag. Accordingly, in some embodiments, provided herein is a host cell expressing a polypeptide of the xylose transporters herein. A polypeptide of the xylose transporters described herein can also be produced by chemical synthesis using a method of polypeptide synthesis well know to one of skill in the art.

[00108] In some embodiments, provided herein are methods of constructing a host strain that can include, among other steps, introducing a vector disclosed herein into a host cell that is capable of fermentation. Vectors of the invention can be introduced stably or transiently into a host cell using techniques well known in the art including, but not limited to, conjugation, electroporation, chemical transformation, transduction, transfection, and

ultrasound transformation. Additional methods are disclosed herein, any one of which can be used in the method of the invention.

[00109] Provided herein are also vectors containing the polynucleotide molecules encoding xylose transporters, as well as host cells transformed with such vectors. Any of the polynucleotide molecules of the disclosure can be contained in a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. The vectors can further include suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, fungal, bacterial, viral, or insect genes, operably linked to the polynucleotide molecule that encode xylose transporter. Examples of such regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding the target protein. Thus, a promoter nucleotide sequence is operably linked to a xylose transporter if the promoter nucleotide sequence directs the transcription of the xylose transporter sequence.

[00110] Selection of suitable vectors for the cloning of nucleic acid molecules encoding the xylose transporter of this disclosure depends upon the host cell in which the vector will be transformed, and, where applicable, the host cell from which the xylose transporter is to be expressed. Suitable host cells for expression of xylose transporter include prokaryotes and yeasts, which are discussed below. Selection of suitable combinations of vectors and host organisms is a routine matter from a perspective of skill.

[00111] The xylose transporter to be expressed in such host cells can also be fusion proteins that include sequences from other proteins. As discussed above, such regions can be included to allow, for example, enhanced functionality, improved stability, or facilitated purification of the xylose transporter. For example, a nucleic acid sequence encoding a peptide that binds strongly to xylose can be fused in-frame to the transmembrane sequence of a xylose transporter so that the resulting fusion protein binds xylose and transports the sugar across the cell membrane at a higher rate than the wild type transporter.

[00112] The non-naturally occurring microbial organisms provided herein can be produced by introducing expressible nucleic acids encoding one or more of the xylose transporters. In some embodiments, the host microbial organisms have one or more biosynthetic pathways

for producing products such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, and 3-methyl-butanol from xylose. The expression of xylose transporters described herein can enhance xylose uptake and increase the production of these bioderived products of these microbial organisms.

5 **[00113]** Host microbial organisms can be selected from, and the non-naturally occurring microbial organisms generated in, for example, bacteria, yeast, fungus or any of a variety of other microorganisms applicable or suitable to fermentation processes. Similarly, exemplary species of yeast or fungi species include any species selected from the order *Saccharomycetales*, family *Saccaromycetaceae*, including the genera *Saccharomyces*,
10 *Debaryomyces*, *Candida, Kluyveromyces* and *Pichia*; the order *Saccharomycetales*, family *Dipodascaceae*, including the genus *Yarrowia*; the order *Schizosaccharomycetales*, family *Schizosaccaromycetaceae*, including the genus *Schizosaccharomyces*; the order *Eurotiales*, family *Trichocomaceae*, including the genus *Aspergillus*; and the order *Mucorales*, family *Mucoraceae*, including the genus *Rhizopus*. Non-limiting species of host yeast or fungi
15 include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida albicans*, *Candida tropicalis*, *Debaryomyces hansenii*, *Kluyveromyces lactis, Kluyveromyces marxianus, Aspergillus terreus, Aspergillus niger*, *Chlamydomonas reinhardtii, Pichia pastoris, Rhizopus arrhizus, Rhizobus oryzae*, *Trichoderma reesei, Yarrowia lipolytica*, and the like.

[00114] The xylose transporters described herein can also be expressed in yeast host cells
20 from genera including *Saccharomyces*, *Pichia*, and *Kluveromyces*. In one embodiment, the yeast host is *S. cerevisiae*. Yeast vectors can contain an origin of replication sequence from a 2μ yeast plasmid for high copy vectors and a CEN sequence for a low copy number vector. Other sequences on a yeast vector can include an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination,
25 and a selectable marker gene. In some embodiments, vectors are replicable in both yeast and bacteria such as *E. coli* (termed shuttle vectors). In addition to the above-mentioned features of yeast vectors, a shuttle vector also includes sequences for replication and selection in bacteria such as *E. coli*.

[00115] Exemplary bacteria include, for example, any species selected from the order
30 *Enterobacteriales*, family *Enterobacteriaceae*, including the genera *Escherichia* and *Klebsiella*; the order *Aeromonadales*, family *Succinivibrionaceae*, including the genus *Anaerobiospirillum*; the order *Pasteurellales*, family *Pasteurellaceae*, including the genera

Actinobacillus and *Mannheimia*; the order *Rhizobiales*, family *Bradyrhizobiaceae*, including the genus *Rhizobium*; the order *Bacillales*, family *Bacillaceae*, including the genus *Bacillus*; the order *Actinomycetales*, families *Corynebacteriaceae* and *Streptomycetaceae*, including the genus *Corynebacterium* and the genus *Streptomyces*, respectively; order *Rhodospirillales*, 5 family *Acetobacteraceae*, including the genus *Gluconobacter*; the order *Sphingomonadales*, family *Sphingomonadaceae*, including the genus *Zymomonas*; the order *Lactobacillales*, families *Lactobacillaceae* and *Streptococcaceae*, including the genus *Lactobacillus* and the genus *Lactococcus*, respectively; the order *Clostridiales*, family *Clostridiaceae*, genus *Clostridium*; and the order *Pseudomonadales*, family *Pseudomonadaceae*, including the 10 genus *Pseudomonas*. Non-limiting species of host bacteria include, for example, *Escherichia coli, Klebsiella oxytoca, Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens, Rhizobium etli, Bacillus subtilis, Corynebacterium glutamicum, Gluconobacter oxydans, Zymomonas mobilis, Lactococcus lactis, Lactobacillus plantarum, Streptomyces coelicolor, Clostridium acetobutylicum,* 15 *Pseudomonas fluorescens*, and *Pseudomonas putida*.

[00116] Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes encode, for example, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include, for example, 20 pSPORT vectors, pGEM vectors (Promega, Madison, Wis.), pPROEX vectors (LTI, Bethesda, Md.), Bluescript vectors (Stratagene), and pQE vectors (Qiagen).

[00117] Insect host cell culture systems can also be used for the expression of the xylose transporters described herein. The target xylose transporters can be expressed using a baculovirus expression system, as described, for example, in the review by Luckow and 25 Summers, 1988.

[00118] *Saccharomyces cerevisiae* is a particularly useful host organism since it is a well characterized microbial organism suitable for genetic engineering. Other particularly useful host organisms include bacteria such as *E. coli*. It is understood that any suitable microbial host organism can be used to express xylose transporters described herein to enhance xylose 30 uptake. The microbial host organism can also be modified to introduce metabolic and/or genetic modifications to produce a desired product or to further enhance the production of a

desired product, such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, and 3-methyl-butanol from xylose.

[00119] The choice of a suitable expression vector for expression of xylose transporters described herein depend upon the host cell to be used. Examples of suitable expression vectors for *E. coli* include pET, pUC, and similar vectors as is known in the art. In some embodiments, the vectors for expression of the xylose transporters include the shuttle plasmid pIJ702 for *Streptomyces lividans*, pGAPZalpha-A, B, C and pPICZalpha-A, B, C (Invitrogen) for *Pichia pastoris*, and pFE-1 and pFE-2 for filamentous fungi and similar vectors as is known in the art.

[00120] Modification of nucleic acids encoding xylose transporters described herein to facilitate insertion into a particular vector (for example, by modifying restriction sites), ease of use in a particular expression system or host (for example, using preferred host codons), and the like, are known and are contemplated for use. Genetic engineering methods for the production of xylose transporters include the expression of the polynucleotide molecules in cell free expression systems, in host cells, in tissues, and in animal models, according to known methods.

[00121] Methods for constructing and testing the expression levels of xylose transporter in a non-naturally occurring host can be performed, for example, by recombinant and detection methods well known in the art. Such methods can be found described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Third Ed., Cold Spring Harbor Laboratory, New York (2001); and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, MD (1999).

[00122] Exogenous nucleic acid sequences involved in a pathway for production of a bioderived product can be introduced stably or transiently into a host cell using techniques well known in the art including, but not limited to, conjugation, electroporation, chemical transformation, transduction, transfection, and ultrasound transformation. For exogenous expression in *E. co*li or other prokaryotic cells, some nucleic acid sequences in the genes or cDNAs of eukaryotic nucleic acids can encode targeting signals such as an N-terminal mitochondrial or other targeting signal, which can be removed before transformation into prokaryotic host cells, if desired. For example, removal of a mitochondrial leader sequence led to increased expression in *E. coli* (Hoffmeister et al., *J. Biol. Chem.* 280:4329-4338

(2005)). For exogenous expression in yeast or other eukaryotic cells, genes can be expressed in the cytosol without the addition of leader sequence, or can be targeted to mitochondrion or other organelles, or targeted for secretion, by the addition of a suitable targeting sequence such as a mitochondrial targeting or secretion signal suitable for the host cells. Thus, it is
5 understood that appropriate modifications to a nucleic acid sequence to remove or include a targeting sequence can be incorporated into an exogenous nucleic acid sequence to impart desirable properties. Furthermore, genes can be subjected to codon optimization with techniques well known in the art to achieve optimized expression of the proteins. Available tools for codon optimization include "UpGene," described in Gao *et al.*, *Biotechnology*
10 *progress* 20.2 (2004): 443-448; "Codon optimizer," described in Fuglsang, *Protein expression and purification* 31.2 (2003): 247-249. As a person of ordinary skill would understand, it would have been a routine practice to use these or any other available tools in the art to codon optimize the specific nucleic acid sequences described herein to express the corresponding gene in a specific host strain.

15 **[00123]** An expression vector or vectors can be constructed to include one or more nucleic acids encoding xylose transporters and/or other enzymes of a biosynthesis pathway operably linked to expression control sequences functional in the host organism. Expression vectors applicable for use in the microbial host organisms of the invention include, for example, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, including
20 vectors and selection sequences or markers operable for stable integration into a host chromosome. Additionally, the expression vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes also can be included that, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression
25 control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more exogenous encoding nucleic acids are to be co-expressed, both nucleic acids can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common
30 expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The transformation of exogenous nucleic acid sequences involved in a metabolic or synthetic pathway can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as

Northern blots or polymerase chain reaction (PCR) amplification of mRNA, or immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the art that the exogenous nucleic acid is expressed in a sufficient amount to produce the desired product, and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art and as disclosed herein.

[00124] Provided herein are also reagents, compositions, and methods that are useful for analysis of xylose transporter activity and for assessing the amount and rate of xylose transport.

[00125] The polypeptide of xylose transporters of the present disclosure, in whole or in part, can be used to raise polyclonal and monoclonal antibodies that are useful in purifying the xylose transporters, or detecting their expression, as well as a reagent tool for characterizing the molecular actions of the xylose transporters. Preferably, a peptide containing a unique epitope of the xylose transporters is used in preparation of antibodies, using conventional techniques. Methods for the selection of peptide epitopes and production of antibodies are known. See, for example, Antibodies: A Laboratory Manual, Harlow and Land (eds.), 1988 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Kennet et al. (eds.), 1980 Plenum Press, New York.

[00126] The non-naturally occurring microbial organisms provided herein have enhanced xylose uptake by expressing xylose transporter described herein. In some embodiments, the microbial organisms provided herein can have one or more biosynthetic pathways to produce compounds such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol from xylose, and the enhanced xylose uptake increases production of such compound. The biosynthetic pathway can be an endogenous pathway or an exogenous pathway. The microbial organisms provided herein can further have expressible nucleic acids encoding one or more of the enzymes or proteins participating in one or more biosynthetic pathways for products such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, and 3-methyl-butanol. Depending on the host microbial organism chosen for biosynthesis, nucleic acids for some or all of a particular biosynthetic pathway can be

expressed. In some embodiments, the host microbial organism can have endogenous expression of all enzymes of a biosynthetic pathway to produce a compound from xylose and naturally produces the compound, which can be improved by further expressing a xylose transporter described herein. In some embodiments, the host microbial organism can be
5 deficient in one or more enzymes or proteins for a desired biosynthetic pathway, then expressible nucleic acids for the deficient enzyme(s) or protein(s) are introduced into the host for subsequent exogenous expression. Alternatively, if the chosen host exhibits endogenous expression of some pathway genes, but is deficient in others, then an encoding nucleic acid is needed for the deficient enzyme(s) or protein(s) to achieve biosynthesis of the desired
10 compound. Thus, a non-naturally occurring microbial organism can further include exogenous enzyme or protein activities to obtain a desired biosynthetic pathway or a desired biosynthetic pathway can be obtained by introducing one or more exogenous enzyme or protein activities that, together with one or more endogenous enzymes or proteins, produces a desired product such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl
15 acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol from xylose.

[00127] Microbial organisms having a biosynthesis pathway to produce xylitol from xylose are known in the art. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing xylitol from
20 xylose. With enhanced xylose uptake the microbial organism can also have improved production of xylitol from xylose. Provided herein are also methods of producing a bioderived xylitol by culturing the non-naturally occurring microbial organism provided herein having a xylitol biosynthesis pathway under conditions and for a sufficient period of time to produce xylitol.

25 **[00128]** Xylitol is a five-carbon sugar alcohol widely used as a low-calorie, low-carbohydrate alternative to sugar; xylitol does not affect insulin levels of people with diabetes and individuals with hyperglycemia (Drucker *et al.*, *Arch of Oral Biol.* 24:965-970 (1979)). Xylitol is approximately as sweet as sucrose but has 33% fewer calories. The consumption of xylitol is also beneficial for dental health as it reduces caries by 33%; xylitol has also been
30 reported to inhibit demineralization of healthy tooth enamel and to re-mineralize damaged tooth enamel (Steinberg *et al.*, *Clinical Preventive Dentistry* 14:31-34 (1992); Maguire *et al.*, *British Dental J.* 194:429-436 (2003); Grillaud *et al.*, *Arch of Pediatrics and Adolescent*

Medicine 12:1180-1186 (2005)). In addition, xylitol in chewing gum inhibits growth of *Streptoccocus mutans* (Haresaku *et al.*, *Caries Res.* 41:198-203 (2007)), and it reduces the incidence of acute middle ear infection (Azarpazhooh *et al.*, *Cochrane Database of Systematic Reviews* 11:CD007095 (2011)).

5 **[00129]** Microbial production of xylitol offers cost effective downstream processing that can reduce manufacturing cost (Rivas *et al.*, *Biotechnol. Prog.* 19:706-713 (2003)). Such process would reduce the need for purified xylose, producing highly pure, easy to separate product, and be adaptable to wide variety of raw material source from different geographical locations (Ur-Rehman *et al.*, *Critical Reviews in Food Science and Nutrition* 55:1514-1528
10 (2013)).

[00130] Many yeast species (*Candida spp.*, *Debaryomyces hansenii*, *Pichia anomala*, *Kluyveromvces spp*, *Pachysolen tannophilus*, *Saccharomyces spp.* and *Schizosaccharomyces pombe*) have been identified with the ability to convert xylose to xylitol (Sirisansaneeyakul *et al.*, *J. Ferment. Bioeng.* 80:565-570 (1995); Onishi *et al.*, *Agric. Biol. Chem.* 30:1139-1144
15 (1966); Barbosa *et al.*, *J. Ind. Microbiol.* 3:241-251 (1988); Gong *et al.*, *Biotechnol. Lett.* 3:125-130 (1981); Vandeska *et al.*, *World J. Microbiol. Biotechnol.* 11:213-218 (1995); Dahiya *et al.*, *Cabdirect.org* 292-303 (1990); Gong *et al.*, *Biotechnol. Bioeng.* 25:85-102 (1983)). The ability to produce xylitol from xylulose has also been discovered in various yeast (*Saccharomyces spp.*, *D. hansenii*, *P. farinose*, *Hansenula spp.*, *Endomycopsis chodatii*,
20 *Candida spp.* and *Cryptococcus neoformans*) (Onishi *et al.*, *Appl. Microbiol.* 18:1031-1035 (1969)). The majority of research into the biological production of xylitol is with yeast, and novel yeast species capable of converting xylose to xylitol continue to be discovered (Kamat *et al.*, *J. App. Microbiol.* 115: 1357-1367 (2013); Bura *et al.*, *J. Ind. Microbiol. Biotechnol.* 39:1003-1011 (2012); Junyapate *et al.*, *Antonie Van Leeuwenhoek* 105:471-480 (2014);
25 Guaman-Burneo *et al.*, *Antonie Van Leeuwenhoek* 108: 919-931 (2015); Cadete *et al.*, *Int. J. Syst. Evolv.* Microbiol. 65:2968-2974 (2015)).

[00131] *S. cerevisiae* is a yeast organism that is used in many food processes, but does not naturally utilize xylose efficiently. It has been engineered to produce xylitol from xylose by expressing xylose reductases from other yeast species such as *S. stipitis (P. stipitis)* and *C.*
30 *shehatae* (Hallborn *et al.*, *Bio/Technology* 9:1090-1095; Hallborn *et al.*, *Appl. Microbiol. Biotechol.* 42:326-333 (1994); Lee *et al.*, *Process Biochem.* 35:1199-1203 (2000); Giovinden

et al., Appl. Microbiol. Biotechnol. 55:76-80 (2001); Chung *et al., Enzyme Microb. Technol.* 30:809-816 (2002)).

[00132] Alternate pathways for xylitol production in *S. cerevisiae* have been explored. Expression of *S. stipitis* xylitol dehydrogenase and deletion of the xylulokinase gene in a
5 transketolase-deficient strain of *S. cerevisiae* allowed conversion of glucose to xylitol through a multistep pathway (Toivari *et al., Appl. Enviorn. Microbiol.* 73:5471-5476 (2007)).

[00133] Expression of *Neurospora crassa* cellodextrin transporter and intracellular β-glucosidase allowed it to simultaneously utilize cellobiose and xylose during xylitol production (Oh *et al., Metab. Eng.* 15:226-234 (2013); Zha *et al., PLoS One* 8:e68317
10 (2013)). Furthermore, the overexpression of *S. cerevisae ALD5*, *IDP2* or *S. stipitis ZWF1* lead to increased NADPH levels, resulting in higher xylitol productivity (Oh *et al., Metab. Eng.* 15:226-234 (2013)).

[00134] Xylitol production can be improved by the use of both NADPH-preferring and NADH-preferring xylose reductases to decrease the limitation of NAD(P)H cofactors. This
15 strategy was used in *S. cerevisiae* with the expression of wild-type NADPH-preferring and mutant NADH-preferring *S. stipitis* xylose reductase and *S. cerevisiae ZWF1* and *ACS1* (Jo *et al., Biotechnol. J.* 10:1935-1943 (2015)).

[00135] In order to decrease processing costs of xylitol production, *S. stipitis* xylose reductase, *Aspergillus aculeatus* β-glucosidase, *A. oryzae* β-xylosidase, and *Trichoderma*
20 *reesei* endoxylanase were expressed in *S. cerevisiae* (Guirimand *et al.*, *Appl. Microbiol. Biotechnol.* 100:3477-3487 (2016)). Expression of these fungal enzymes allowed direct degradation of hemicellulose without the addition of exogenous enzymes.

[00136] *C. tropicalis* is pathogenic, but is also one of the natural producers of xylitol. Several patents and literature have described the application of yeast from genus *Candida* as
25 the host strain for xylitol production from xylose; *i.e.* *C. tropicalis* ATCC 13803 (PCT/IN2009/000027 & KR100259470), *C. tropicalis* ATCC 9968 (PCT/FI1990/000015), *C. tropicalis* KFCC 10960 (KR100199819), *C. tropicalis* (NRRL 12968) (PCT/IN2013/000523), *C.tropicalis* ATCC 750 (West *et al.*, *World J. Mircrobiol. Biotechnol.* 25:913-916 (2009)) and *C. tropicalis* ATCC 7349 (SAROTE *et al.*, *J. Ferment. and Bioeng.*
30 80:565-570 (1995)). One strategy used to improve xylitol production in *C. tropicalis* was the

expression of an NADH-preferring xylose reductase from *C. parapsilosis*, which allowed reduction of xylose with both NADPH and NADH (Lee *et al., Appl. Enviorn. Microbiol.* 69:6179-6188 (2003)). Deletion of xylitol dehydrogenase increases xylitol production by blocking xylitol catabolism, but a co-substate such as glucose or glycerol is needed to
5 regenerate NADPH for xylose reductase activity (Ko *et al., Appl. Environ. Microbiol.* 72:4207-4213 (2006); Ko *et al., Biotechnol. Lett.* 28:1159-1162 (2006)). Further improvements for xylitol production were made by combining deletion of the xylitol dehydrogenase gene with expression of *N. crassa* xylose reductase (Jeon *et al., Bioprocess Biosyst. Eng.* 35:191-198 (2012)). The xylose uptake and xylitol productivity of this strain
10 was again further improved by expressing a xylose transporter from *Arabidopsis thaliana* (Jeon *et al., Bioprocess Biosyst. Eng.* 36:809-817 (2013)).

[00137] If glycerol is provided as a co-substrate, NADPH regeneration can be enhanced by expressing glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in *C. tropicalis* (Ahmad *et al., Bioprocess Biosyst. Eng.* 35:199-204 (2012)). Xylitol production
15 can also be enhanced by deleting glycerol kinase and expressing three NADPH-regenerating glycerol dehydrogenases from *S. stipitis* (Ahmad *et al., Bioprocess Biosyst. Eng.* 36:1279-1284 (2013)). One of the problems with producing xylitol from mixed sugar substrates is that the xylose reductase from *C. tropicalis* can convert arabinose to arabitol, a contaminant in xylitol production. To prevent this, the endogenous xylose reductase was deleted and a
20 mutant xylose-specific xylose reductase from *N. crassa* was expressed along with bacterial arabinose assimilation enzymes (Yoon *et al., Biotechnol. Lett.* 33:747-753 (2011); Nair *et al., ChemBioChem* 9:1213-1215 (2008)). This minimized arabitol formation while allowing arabinose assimilation for cell growth.

[00138] *K. marxianus* is a thermotolerant yeast often found in dairy products. It can be
25 used for xylitol production due to its high growth rate, tolerance to temperatures up to 52 °C, and ability to utilize various sugars. Expression of the *N. crassa* xylose reductase alone or in conjunction with deletion of the xylitol dehydrogenase gene in *K. marxianus* led to xylitol production optimally at 42 °C (Zhang *et al., Bioresour. Technol.* 152:192-201 (2014)). Further improvements to xylitol production were made by testing the expression of various
30 xylose transporters: *K. marxianus* aquaglyceroporin, *C. intermedia* glucose/xylose facilitator, or *C. intermedia* glucose/xylose symporter (Zhang *et al., Bioresour. Technol.* 175:642-645 (2015)). The expression of the *C. intermedia* glucose/xylose facilitator was found to be

effective at increasing xylitol yield and productivity, and notably, produced the highest reported final xylitol concentration. *K. marxianus* was also used in an evolutionary adaptation experiment that resulted in a strain with improved xylose utilization and xylitol production capabilities (Sharma *et al., Bioprocess Biosyst. Eng.* 39:835-843 (2016)).

5 **[00139]** Two other yeast species have been genetically engineered to explore xylitol production. *D. hansenii* is another natural producer of xylitol that is osmotolerant and non-pathogenic. Xylitol production was enhanced in this species by deletion of the xylitol dehydrogenase gene (Pal *et al., Bioresour. Technol.* 147:449-455 (2013)). *P.pastoris* is a yeast commonly used for protein expression. It has been engineered to produce xylitol 10 directly from glucose through the glucose–arabitol–xylulose–xylitol pathway (Cheng *et al., Appl. Microbiol. Biotechnol.* 98:3539-3552 (2014)). This was achieved by expressing xylitol dehydrogenase from *Gluconobacter oxydans* and the xylulose-forming arabitol dehydrogenase from *Klebsiella pneumoniae*.

[00140] In addition to filamentous fungi and yeast, a limited number of bacterial species 15 (*Corynebacterium sp.* and *Enterobacter liquefaciens*) have been observed to produce xylitol from xylose (Yoshitake *et al., Agric. Biol. Chem.* 35:905-911 (1971); Yoshitake *et al., Agric. Biol. Chem.* 37:2261-2267 (1973); Yoshitake *et al., Agric. Biol. Chem.* 40:1493-1503 (1976); Rangaswamy *et al., Appl. Microbiol. Biotechnol.* 60:88-93 (2002)). *Mycobacterium smegmatis* has also been reported to be able to produce xylitol from xylulose (Izumori *et al.,* 20 *J. Ferment. Technol.* 66:33-36 (1988)). A subsequent screen of bacteria discovered that *Gluconobacter spp.* and *Acetobacter xylinum* are capable of converting arabitol to xylitol through the sequential conversion of arabitol to xylulose and xylulose to xylitol (Suzuki *et al., Biosci. Biotechnol. Biochem.* 66:2614-2620 (2002)).

[00141] Microalgae are an attractive platform for the production of renewable resources. 25 Xylitol production in microalgae has been reported once, where expression of the xylose reductase from *Neurospora crassa in Chlamydomonas reinhardtii* allowed it to convert a small amount of xylose to xylitol (Pourmir *et al., J. Biotechnol.* 165:178-183 (2013)).

[00142] The extracts of various filamentous fungi (*Penicillium spp.*, *Aspergillus spp.*, *Rhizopus nigricans*, *Gliocladium roseum*, *Byssochlamys fulva*, *Myrothecium verrucaria*, 30 *Neurospora crassa*, *Rhodotorula glutinis* and *Torulopsis utilis*) have been observed to contain an enzyme capable of converting xylose to xylitol (Chiang *et al.*, *Nature* 188:79-81

(1960); Chiang *et al.*, *Biochem. Biophys. Res. Commun.* 3:554-559 (1960); Chiang *et al.*, *Biochem. Biophys. Acta.* 29:664-5 (1958)). Subsequent studies identified additional filamentous fungi (*Petromyces albertensis*, *Penicillium spp.* and *A. niger*) capable of converting xylose to xylitol with varying degrees of efficiency (Dahiya *et al.*, *Can. J.* 5 *Microbiol.* 37:14-18 (1991); Sampaio *et al.*, *Brazilian J. Microbiol.* 34:325-328 (2003)).

[00143] *Trichoderma reesei*, a filamentous fungus that secretes celluloytic enzymes, produced more xylitol when the genes for xylitol dehydrogenase and L-arabinitol-4-dehydrogenase were deleted in order to block xylitol metabolism (Dashtban *et al., Appl. Biochem. Biotecnol.* 169:554-569(2013)). Xylitol production also increased in *T. reesei* 10 when xylose reductase was overexpressed and xylulokinase was inhibited (Hong *et al., Biomed Res. Int.* 2014:169705 (2014)). *Phanerochaete sordida*, a white-rot fungus with ligninolytic activity, produced more xylitol when it expressed the xylose reductase gene from *P. chrysosporium* (Hirabayashi *et al.*, *J. Biosci. Bioeng.* 120:6-8 (2015)).

[00144] Bacteria metabolize xylose with xylose isomerases instead of with the xylose 15 reductase-xylitol dehydrogenase pathway. Therefore, the use of bacterial hosts for xylitol production typically involves recombinant expression of xylose reductases. Xylose reductase from *C. tropicalis* was expressed in *E. coli* and was found to be functional for xylitol production from xylose (Suzuki *et al., J. Biosci. Bioeng.* 87:280-284 (1999)). A subsequent study expressed xylose reductases from *C. boidinii*, *C. tenuis* and *S. stipitis* in conjunction 20 with a deletion of the endogenous xylulokinase gene (Cirino *et al., Biotechnol. Bioeng.* 95:1167-1176 (2006)). In order to improve xylitol production from mixtures of glucose and xylose, the cyclic AMP receptor protein was replaced with a mutant that circumvents glucose repression of xylose metabolism. Expressing the xylose transporters, XylE or XylFGH, has similar effects to replacing the cyclic AMP receptor protein with a mutant form (Khankal *et* 25 *al., J. Biotechnol.* 134:246-252 (2008)).

[00145] Cofactor regeneration is also important for improving xylitol production in bacteria, which has been explored in *E. coli* through a large number of gene deletions and expression of cofactor regenerating pathways (Chin *et al., Biotechnol. Bioeng.* 102:209-220 (2009); Chin *et al., Biotechnol. Prog.* 27:333-341 (2011); Iverson *et al., World J. Microbiol.* 30 *Biotechnol.* 29:1225-1232 (2013); Iverson *et al., BMC Syst. Biol.* 10:31 (2016)). Another study aimed at improving xylitol production from mixtures of glucose and xylose disrupted the phosphoenolpyruvate-dependent glucose phosphotransferase system to eliminate

catabolite repression (Su *et al., Metab. Eng.* 31:112-122 (2015)). Endogenous xylose metabolism was blocked in this strain by disrupting xylose isomerase, xylulose kinase, and the phosphoenolpyruvate-dependent fructose phosphotransferase system, and the *N. crassa* xylose reductase was expressed to optimize xylitol production.

5 **[00146]** *L. lactis* is a well-characterized bacterium commonly used for dairy processes such as cheese production, and could be adopted for other food-related processes. *L. lactis* was able to produce xylitol from xylose when it expressed the *S. stipitis* xylose reductase and the *L. brevis* xylose transporter (Nyyossola *et al., J. Biotechnol.* 118:55-56 (2005)).

[00147] *C. glutamicum* is a bacterium with many industrial uses such as the production of 10 MSG. It has been engineered to co-utilize xylose and glucose, which is an important trait for xylitol productivity (Sasaki *et al., Appl. Microbiol. Biotechnol.* 85:105-115 (2009)). To optimize xylitol production in *C. glutamicum*, it has been engineered to express a pentose transporter and a mutant xylose reductase from *C. tenuis* in conjunction with disruptions of its lactate dehydrogenase, xylulokinase, and phosphoenolpyruvate-dependent fructose 15 phosphotransferase genes (Sasaki *et al., Appl. Microbiol. Biotechnol.* 86:1057-1066 (2010)). Xylitol production in *C. glutamicum* was also achieved by expressing *S. stipitis* xylose reductase (Kim *et al., Enzyme Microb. Technol.* 46:366-371 (2010)). Expression of *Rhodotorula mucilaginosa* xylose reductase, *E. coli* l-arabinose isomerase, *Agrobacterium tumefaciens* d-psicose-3-epimerase, *Mycobacterium smegmatis* l-xylulose reductase, and a 20 fusion pentose transporter allowed the production of xylitol from mixtures of xylose and arabinose without the formation of arabitol (Dhar *et al., J. Biotechnol.* 230:63-71 (2016)).

[00148] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce xylitol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved 25 production of xylitol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase xylitol production in these host strains.

[00149] Microbial organisms having a biosynthesis pathway to produce ethanol from xylose are known in the art. In some embodiments, provided herein are non-naturally 30 occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing ethanol from

xylose. With enhanced xylose uptake the microbial organism can also have improved production of ethanol from xylose. Provided herein are also methods of producing a bioderived ethanol by culturing the non-naturally occurring microbial organism provided herein having an ethanol biosynthesis pathway under conditions and for a sufficient period of
5 time to produce ethanol.

[00150] Ethanol has a number of uses and is most commonly used as a fuel additive. As a fuel additive, ethanol is a low value product with much of the cost of its production attributed to the cost of raw materials. It would be desirable, therefore, to develop ethanologens and fermentation processes for the production of ethanol from readily available, inexpensive
10 starting materials, such as lignocellulose. Fermentation of both glucose and xylose is currently regarded as a high priority for economical conversion of biomass into ethanol. Most microorganisms are able to ferment glucose but few have been reported to utilize xylose efficiently and even fewer ferment this pentose to ethanol.

[00151] A relatively small number of wild type microorganisms can ferment D-xylose.
15 These microorganisms are generally not suitable for large-scale fermentation. This unfavorability may arise, for example, as a result of unfamiliarity with the microorganisms, difficulty obtaining the microorganisms, poor productivity and/or growth on pretreated lignocellulosics or unsatisfactory yield when grown on mixed sugars derived from biomass. (C. Abbas, "Lignocellulosics to ethanol: meeting ethanol demand in the future," The Alcohol
20 Textbook, 4th Edition. (K. A. Jacques, T. P. Lyons and D. R. Kelsall, eds). Nottingham University Press, Nottingham, UK, 2003, pp. 41-57.; C. Abbas, "Emerging biorefineries and biotechnological applications of nonconventional yeast: now and in the future," The Alcohol Textbook, 4th Edition. (K. A. Jacques, T. P. Lyons and D. R. Kelsall, eds). Nottingham University Press, Nottingham, United Kingdom, 2003, pp. 171-191).

25 **[00152]** Yeasts are considered promising microorganisms for alcoholic fermentation of xylose (see Ryabova, supra). They have larger cells than bacteria, are resistant to viral infection, and tend to be more resistant to negative feedback from ethanol. Furthermore, yeast growth and metabolism have been extensively studied for a number of species.

[00153] A number of yeasts are known to naturally ferment D-xylose. These include, for
30 example, *P. stipitis, C. shehatae*, and *P. tannophilus* (see Ryabova, supra; Cite 2, C. Abbas 2003). The common brewer's yeast *S. cerevisiae* is not known to ferment D-xylose naturally,

but a number of strains of metabolically engineered *S. cerevisiae* that do ferment D-xylose have been reported.

[00154] Numerous studies have described the metabolism of D-xylose by recombinant *S. cerevisiae* (*see, e.g.*, Matsushika *et al.*, *Applied Microbiology and Biotechnology* 84, no. 1 (2009): 37-53; U.S. Pat. Pub. No. 2005/0153411A1 (Jul. 14, 2005); U.S. Pat. Pub. No. 2004/0231661A1 (Nov. 25, 2004); U.S. Pat. No. 4,368,268 (Jan. 11, 1983); U.S. Pat. No. 6,582,944 (Jun. 24, 2003); U.S. Pat. No. 7,226,735 (Jun. 5, 2007); U.S. Pat. Pub. No. 2004/0142456A1 (Jul. 22, 2004); Jeffries, T. W. & Jin, Y-S., *Appl. Microbiol. Biotechnol.* 63: 495-509 (2004); Jin, Y-S., *Met. Eng.* 6: 229-238 (2004); Pitkanen, J-Y., Helsinki Univ. of Tech., Dept. of Chem. Tech., Technical Biochemistry Report (January 2005); Porro, D. *et al.*, *App. & Env. Microbiol.* 65(9): 4211-4215 (1999); Jin, Y-S., et al., *App. & Env. Microbiol.* 70(11): 6816-6825 (2004); Sybirna, K, et al., *Curr. Genetics* 47(3): 172-181 (2005); Toivari, M. H., *et al.*, *Metabolic Eng.* 3:236-249 (2001).

[00155] D-Xylose metabolism in yeast proceeds along a pathway similar to that of glucose via pentose phosphate pathway. Carbon from D-xylose is processed to ethanol via the glycolytic cycle or to CO_2 via respiratory TCA cycle. Fermentation to ethanol relies in part on the metabolism of pyruvate, which is a metabolite that may be used in either respiration or fermentation (see van Hoek, P., *et al.*, *Appl. & Enviro. Microbiol.* 64(6); 2133-2140 (1998)). Pyruvate enters fermentation following decarboxylation of pyruvate to acetaldehyde by the enzyme pyruvate decarboxylase (E.C. 4.1.1.1). Pyruvate decarboxylase is a member of the family of biotin-dependent carboxylases. It catalyzes the decarboxylation of pyruvate to form oxaloacetate with ATP cleavage. The oxaloacetate can be used for synthesis of fat, glucose, and some amino acids or other derivatives. The enzyme is highly conserved and found in a variety of prokaryotes and eukaryotes.

[00156] Other microbial organisms capable of ethanol production from xylose are also known in the art. The thermotolerant methylotrophic yeast *Hansenula polymorpha* (also known as *P. angusta*) was reported to have optimum and maximum growth temperatures of 37° C. and 48° C., respectively, and can naturally ferment D-xylose under certain conditions. (US 8071298; Voronovsky *et al.*, *FEMS Yeast Res.* 5(11): 1055-62 (2005)). Additionally, three strains of *P. stipitis* and three of *C. shehatae* were reported to ferment xylose when subjected to both aerobic and microaerophilic conditions. Of the strains considered, *P. stipitis* NRRL Y-7124 was able to utilize all but 7 g/L of 150 g/L xylose supplied aerobically

to produce 52 g/L ethanol at a yield of 0.39 g per gram xylose (76% of theoretical yield) and at a rate comparable to the fastest shown by *C. shehatae* NRRL Y-12878. For all strains tested, fermentation results from aerobic cultures were more favorable than those from microaerophilic cultures. Slininger, P.J. *et al.*, *Biotechnol Lett* (1985) 7: 431.

5 **[00157]** For example, *Zymomonas mobilis*, a bacterial ethanologen that grows on glucose, fructose, and sucrose, metabolizing these sugars to CO_2 and ethanol via the Entner-Douderoff pathway. Though wild type strains cannot use xylose as a carbon source, recombinant strains of Z. mobilis that are able to grow on this sugar have been engineered (U.S. patent publication No. 20080187973, U.S. Pat. No. 5,514,583, U.S. Pat. No. 5,712,133, WO 10 95/28476, Feldmann et al. (1992) *Appl Microbiol Biotechnol* 38: 354-361, Zhang et al. (1995) *Science* 267:240-243).

[00158] The conversion of xylose to ethanol by recombinant *E. coli* has been reported. The addition of small amounts of calcium, magnesium, and ferrous ions stimulated fermentation. Beall *et al.*, *Biotechnology and Bioengineering* 38, no. 3 (1991): 296-303.

15 **[00159]** It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce ethanol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of ethanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to 20 further increase ethanol production in these host strains.

[00160] Microbial organisms having a biosynthesis pathway to produce n-butanol from xylose are known in the art. *See e.g.* Kudahettige-Nilsson RL, *et al.*, *Bioresour Technol.* 176:71-9 (2015); Xin F, *et al.*, *Appl Environ Microbiol.*, 80(15):4771-8 (2014); Xiao H, *et al.*, *Metab Eng.* 14(5):569-78 (2012); Zhang J, *et al.*, *Biotechnol Lett.* 38(4):611-7 (2016); Yu L, 25 *et al. Biotechnol Bioeng.* 112(10):2134-41 (2015); Steen, *et al, Microb Cell Fact.* 7:36 (2008); *Pásztor A, et al., Biotechnol Bioeng.,* 112(1):120-8 (2015); Shi S, *et al., Sci Rep.* 6:25675(2016); Dellomonaco C, *et al., Nature,* 10:476(7360):355-9 (2011). In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as 30 a biosynthesis pathway for producing n-butanol from xylose. With enhanced xylose uptake the microbial organism can also have improved production of n-butanol from xylose.

Provided herein are also methods of producing a bioderived n-butanol by culturing the non-naturally occurring microbial organism provided herein having a n-butanol biosynthesis pathway under conditions and for a sufficient period of time to produce n-butanol.

[00161] Butanol offers a number of benefits as a fuel. Butanol is a four-carbon alcohol, a clear neutral liquid miscible with most solvents (alcohols, ether, aldehydes, ketones and hydrocarbons) and is sparingly soluble in water (water solubility 6.3% as compared to ethanol which is totally miscible). It has an octane rating comparable to gasoline, making it a valuable fuel for any internal combustion engine made for burning gasoline. Fuel testing also has proven that butanol does not phase separate in the presence of water, and has no negative impact on elastomer swelling. Butanol not only has a higher energy content that is closer to that of gasoline than ethanol, so it is less of a compromise on fuel economy, but it also can be easily added to conventional gasoline due to its low vapor pressure.

[00162] Butanol biosynthesis can be achieved through the acetone, butanol, and ethanol fermentation pathway (the "ABE pathway"). The products of this butanol fermentative production pathway using a solvent-producing species of the bacterium *Clostridium acetobutylicum* are six parts butanol, three parts acetone, and one part ethanol. Butanol-production pathway has been introduced to various host organisms. For instance, the pathway was expressed in *Escherichia coli* (Atsumi *et al.*, *Nature* 451:86-89 (2008)) and *S. cerevisiae* (Steen *et al.*, *Microb. Cell Fact* 7:36 (2008)) for their high growth rates and the efficiency of genetic tools. *P. putida*, *L. brevis* and *B.subtilis* were used for their potentially higher solvent tolerance (Nielsen *et al.*, *Metab. Eng.* 11:262-273 (2009); Berezina *et al.*, *Appl. Microbiol. Biot.* 87:635-646 (2010)).

[00163] An alternative to the use of food crops as starting material for butanol production is biomass, specifically lignocellulosic biomass. *Clostridium spp.* strains have been engineered to produce butanol for xylose, such as *C. saccharoperbutylacetonicum* (*e.g.*, *C. saccharoperbutylacetonicum* strain ATCC 27021 or *C. saccharoperbutylacetonicum* strain ATCC 27022). *See e.g.* U.S. Patent No. 8900841. *C. cellulolyticum* was engineered to divert its native valine synthesis pathway for isobutanol production from crystalline cellulose (Higashide *et al.*, *Appl. Environ. Microb.* 77:2727-2733 (2011)). *C. cellulovorans*, which natively produces butyric acid as the main metabolic product, was introduced with an aldehyde/alcohol dehydrogenase (AdhE2) to convert precursor butyryl-CoA to 1-butanol from cellulose (Yang *et al.*, *Metab. Eng.* 32:39-48 (2015)). 1-Butanol production from xylose

was also demonstrated using *Thermoanaerobacterium saccharolyticum* (Bhandiwad *et al.*, *Metab. Eng.* 21:17-25 (2014)).

[00164] To increase the cellulose decomposition rate and to reduce chance of contamination, thermophilic organisms were used. The first example of isobutanol production 5 in thermophiles was demonstrated in *Geobacillus thermoglucosidasius* using cellobiose as substrate (Lin *et al.*, *Metab. Eng.* 24:1-8 (2014)). In this work, thermostabilities of enzymes involved in isobutanol synthesis were investigated. The result of this study was applied to the direct conversion of cellulose to isobutanol in *C. thermocellum* by expressing and optimizing the isobutanol biosynthesis pathway (Lin *et al.*, *Metab. Eng.* 31:44-52 (2015)).

10 **[00165]** *S. cerevisiae* has several benefits such as high ethanol production from hexoses and high tolerance to ethanol and other inhibitory compounds in the acid hydrolysates of lignocellulose biomass. Although standard strains of this yeast cannot utilize pentoses, such as xylose, a recombinant yeast strain can be provided that can ferment xylose and cellooligosaccharides by integrating genes for the intercellular expression of xylose 15 assimilation pathways, such as xylose reductase and xylitol dehydrogenase from *P. stipitis* and a gene for displaying β-glucosidase from *A. acleatus*. *See e.g.* U.S. Patent Publication No. 20100129885; U.S. Patent Publication No. 20100261241.

[00166] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce n-butanol from 20 xylose can be used as the host strain. These microbial organisms can have enhanced xylose uptake and improved production of n-butanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase n-butanol production in these host strains.

[00167] Microbial organisms having a biosynthesis pathway to produce isobutanol from 25 xylose are known in the art. *See e.g.* Felpeto-Santero C, *et al.*, *AMB Express* 5(1):119 (2015). In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing isobutanol from xylose. With enhanced xylose uptake the microbial organism can also have improved production of isobutanol from 30 xylose. Provided herein are also methods of producing a bioderived isobutanol by culturing the non-naturally occurring microbial organism provided herein having a isobutanol

biosynthesis pathway under conditions and for a sufficient period of time to produce isobutanol.

[00168] Isobutanol, also a biofuel candidate, has been produced in recombinant microorganisms expressing a heterologous, five-step metabolic pathway (*See, e.g.*, WO/2007/050671, WO/2008/098227, and WO/2009/103533). Other pathways for isobutanol production are also known in the art. *See e.g.*, US 8530226 B2; US 8114641 B2; US 8975049 B2. The recombinant microorganism including a pathway for the production of isobutanol from five-carbon (pentose) sugars including xylose is also known in the art. (*See e.g.*, WO 2012173659; WO 2011153144). The recombinant microorganism can be engineered to express a functional exogenous xylose isomerase. Exogenous xylose isomerases functional in yeast are known in the art. *See, e.g.*, US2006/0234364. The exogenous xylose isomerase gene can be operatively linked to promoter and terminator sequences that are functional in the yeast cell. Various methods of genetic engineering to improve isobutanol production are also known in the art. (*See e.g.*, Avalos *et al.*, *Nature Biotechnology* 31, 335–41 (2013).)

[00169] For example, recombinant *S. cerevisiae* was known to produce isobutanol from xylose. *See e.g.* US20130035515, Brat *et al.*, *FEMS yeast research* 13.2 (2013): 241-244; Lee, Won-Heong *et al. Bioprocess and biosystems engineering* 35.9 (2012): 1467-1475; Simultaneous overexpression of an optimized, cytosolically localized valine biosynthesis pathway together with overexpression of xylose isomerase XylA from *C. phytofermentans*, transaldolase Tal1 and xylulokinase Xks1 enabled recombinant *S. cerevisiae* cells to complement the valine auxotrophy of *ilv2,3,5* triple deletion mutants for growth on D-xylose as the sole carbon source. Moreover, after additional overexpression of ketoacid decarboxylase Aro10 and alcohol dehydrogenase Adh2, the cells were able to ferment D-xylose directly to isobutanol.

[00170] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce isobutanol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of isobutanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase isobutanol production in these host strains.

[00171] Microbial organisms having a biosynthesis pathway to produce isopropanol are known in the art. Hanai T, *et al.*, *Appl Environ Microbiol.*, 73(24):7814-8 (2007). In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing isopropanol from xylose. With enhanced xylose uptake the microbial organism can also have improved production of isopropanol from xylose. Provided herein are also methods of producing a bioderived isopropanol by culturing the non-naturally occurring microbial organism provided herein having an isopropanol biosynthesis pathway under conditions and for a sufficient period of time to produce isopropanol.

[00172] Polymerization of ethylene provides polyethylene, a type of plastic with a wide range of useful applications. Ethylene is traditionally produced by refined non-renewable fossil fuels, but dehydration of biologically-derived ethanol to ethylene offers an alternative route to ethylene from renewable carbon sources, *i.e.*, ethanol from fermentation of fermentable sugars. Similarly, isopropanol and n-propanol can be dehydrated to propylene, which in turn can be polymerized to polypropylene. As with polyethylene, using biologically-derived propanol starting material (*i.e.*, isopropanol or n-propanol) would result in "Green Polypropylene." *See e.g.* WO 2009/049274, WO 2009/103026, WO 2009/131286, WO 2010/071697, WO 2011/031897, WO 2011/029166, WO 2011/022651 , WO 2012/058603.

[00173] Production of isopropanol has been observed in recombinant *Lactobacillus* host cells (*e.g.*, *Lactobacillus reuteri*) engineered to have an isopropanol pathway and produce increased amounts of isopropanol. *See e.g.* WO2013178699 A1. Direct isopropanol production from cellobiose by engineered *Escherichia coli* using a synthetic pathway was also observed. *See e.g.* Soma *et al.*, *Journal of bioscience and bioengineering* 114.1: 80-85 (2012).

[00174] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce isopropanol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of isopropanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase isopropanol production in these host strains.

[00175] Arabitol belongs to the pentitol family and is used in the food industry as a sweetener and in the production of human therapeutics as an anticariogenic agent and an adipose tissue reducer. It can also be utilized as a substrate for chemical products such as arabinoic and xylonic acids, propylene, ethylene glycol, xylitol and others. It is included on
5 the list of 12 building block C3-C6 compounds, designated for further biotechnological research. This polyol can be produced by yeasts in the processes of bioconversion or biotransformation of waste materials from agriculture, the forest industry (L-arabinose, glucose) and the biodiesel industry (glycerol). There are native yeasts from the genera *Candida*, *Pichia*, *Debaryomyces* and *Zygosaccharomyces* as well as genetically modified
10 strains of *Saccharomyces cerevisiae* that are able to utilize biomass hydrolysates to effectively produce L- or D-arabitol. Kordowska-Wiater, *Journal of Applied Microbiology* 119, 303-314 (2015).

[00176] Microbial organisms having a biosynthesis pathway to produce arabitol are known in the art. (*See e.g.* Kordowska-Wiater, *Journal of Applied Microbiology* 119, 303-314
15 (2015); Nozaki *et al.*, *Biosci. Biotechnol. Biochem.*, 67(9): 1923-29 (2003).) For example, the recently identified *Zygocaccharomyces rouxxii NRRL 27,624* strain is known to produce D-arabitol as the main metabolic product from glucose (Saha *et al.*, *J Ind Microbiol Biotechnol* 34:519–523 (2007)). However, it also was identified as producing D-arabitol and xylitol from xylose and from a mixture of xylose and xylulose (Saha *et al.*, 2007). Based on
20 these results, the pathway for production of D-arabitol from xylose included a xylose reductase, a xylitol dehydrogenase and an arabitol dehydrogenase (Saha *et al.*, 2007). Additionally, *Candida maltosa* has been shown to produce D-arabitol from D-xylulose by a xylulose reductase (Cheng *et al.*, Microbial. Cell Factories, 10:5 (2011)). Production of arabitol was also found to be improved by the addition of xylose with glycerol in the yeast
25 species within the genus of *Debaryomyces*, *Geotrichum* and *Metschnikowia* (International Application Publication WO 2012/011962 (2012)).

[00177] In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing arabitol from xylose. With
30 enhanced xylose uptake the microbial organism can also have improved production of arabitol from xylose. Provided herein are also methods of producing a bioderived arabitol by

culturing the non-naturally occurring microbial organism provided herein having an arabitol biosynthesis pathway under conditions and for a sufficient period of time to produce arabitol.

[00178] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce arabitol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of arabitol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase arabitol production in these host strains.

[00179] Microbial organisms having a biosynthesis pathway to produce ethyl acetate from xylose are known in the art. Morrissey JP, *et al.*, *Yeast*, 32(1):3-16 (2015). In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing ethyl acetate from xylose. With enhanced xylose uptake the microbial organism can also have improved production of ethyl acetate from xylose. Provided herein are also methods of producing a bioderived ethyl acetate by culturing the non-naturally occurring microbial organism provided herein having an ethyl acetate biosynthesis pathway under conditions and for a sufficient period of time to produce ethyl acetate.

[00180] Ethyl acetate is an environmentally friendly solvent with many industrial applications. Microbial synthesis of ethyl acetate is desirable. The ability of yeasts for producing larger amounts of this ester is known for a long time and can be applied to large-scale ester production from renewable raw materials. *P. anomala*, *C. utilis*, and *K. marxianus* are yeasts which convert sugar into ethyl acetate with a high yield. Löser *et al.*, *Appl Microbiol Biotechnol* (2014) 98:5397–5415.

[00181] Synthesis of much ethyl acetate requires oxygen which is usually supplied by aeration. Ethyl acetate is highly volatile so that aeration results in its phase transfer and stripping. This stripping process cannot be avoided but requires adequate handling during experimentation and offers a chance for a cost-efficient process-integrated recovery of the synthesized ester.

[00182] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce ethyl acetate from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of ethyl acetate from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase ethyl acetate production in these host strains.

[00183] Microbial organisms having a biosynthesis pathway to produce phenyl-ethyl alcohol are known in the art. *See e.g.* Kim B, *et al.*, *Biotechnol Bioeng.* 111(1):115-24 (2014). In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing phenyl-ethyl alcohol from xylose. With enhanced xylose uptake the microbial organism can also have improved production of phenyl-ethyl alcohol from xylose. Provided herein are also methods of producing a bioderived phenyl-ethyl alcohol by culturing the non-naturally occurring microbial organism provided herein having an phenyl-ethyl alcohol biosynthesis pathway under conditions and for a sufficient period of time to produce phenyl-ethyl alcohol.

[00184] Phenyl-ethyl alcohol a colorless, transparent, slightly viscous liquid that can be produced by microbial organisms. Phenyl-ethyl alcohol has been found in a number of natural essential oils, in food, spices and tobacco, and in undistilled alcoholic beverages, beers and wines. It prevents or retards bacterial growth, and thus protects cosmetics and personal care products from spoilage. Phenyl-ethyl alcohol also imparts a fragrance to a product.

[00185] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce phenyl-ethyl alcohol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of phenyl-ethyl alcohol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase phenyl-ethyl alcohol production in these host strains.

[00186] Microbial organisms having a biosynthesis pathway to produce 2-methyl-butanol are known in the art. *See e.g.* US 8114641 B2; US 8975049 B2. In some embodiments,

provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing 2-methyl-butanol from xylose. With enhanced xylose uptake the microbial organism can also have improved production of 2-methyl-butanol from
5 xylose. Provided herein are also methods of producing a bioderived 2-methyl-butanol by culturing the non-naturally occurring microbial organism provided herein having a 2-methyl-butanol biosynthesis pathway under conditions and for a sufficient period of time to produce 2-methyl-butanol.

[00187] 2-methyl-butanol can be used as a solvent and an intermediate in the manufacture
10 of other chemicals. 2-methyl-butanol also has applications in fuel and lubricating oil additives, flotation aids, manufacture of corrosion inhibitors, pharmaceuticals, paint solvent, and extraction agent.

[00188] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce 2-methyl-butanol
15 from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of 2-methyl-butanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase 2-methyl-butanol production in these host strains.

[00189] Microbial organisms having a biosynthesis pathway to produce 3-methyl-butanol
20 are known in the art. *See e.g.* US 8114641 B2; US 8975049 B2; US 7985567 B2. In some embodiments, provided herein are non-naturally occurring microbial organisms having at least one exogenous nucleic acid encoding a xylose transporter as described herein, as well as a biosynthesis pathway for producing 3-methyl-butanol from xylose. With enhanced xylose uptake the microbial organism also has improved production of 3-methyl-butanol from
25 xylose. Provided herein are also methods of producing a bioderived 3-methyl-butanol by culturing the non-naturally occurring microbial organism provided herein having a 3-methyl-butanol biosynthesis pathway under conditions and for a sufficient period of time to produce 3-methyl-butanol.

[00190] 3-methyl-butanol (also known as isoamyl alcohol or isopentyl alcohol) is a clear,
30 colorless alcohol. 3-methyl-butanol is a main ingredient in the production of banana oil, an ester found in nature and also produced as a flavouring in industry. It is also the main

ingredient of Kovac's reagent, used for the bacterial diagnostic indole test. 3-methyl-butanol is also used as an antifoaming agent in the chloroform:isomyl alcohol reagent.

[00191] It is understood that microbial organisms provided herein or otherwise known in the art with either natural or engineered biosynthesis pathways to produce 3-methyl-butanol from xylose can be used as the host strain, which can have enhanced xylose uptake and improved production of 3-methyl-butanol from xylose when expressing an exogenous nucleic acid encoding a xylose transporter as described herein. Further metabolic engineering can be adopted to further increase 3-methyl-butanol production in these host strains.

[00192] Depending on the biosynthetic pathway constituents of a selected host microbial organism for a particular compound, the non-naturally occurring microbial organisms provided herein can include at least one exogenously expressed biosynthetic pathway-encoding nucleic acid and up to all encoding nucleic acids for one or more biosynthetic pathways of the compound. The compound can be, for example, xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. For example, ethanol biosynthesis can be established in a host deficient in a pathway enzyme or protein that is required to produce ethanol from xylose through exogenous expression of the corresponding encoding nucleic acid. In other words, in a host deficient in all enzymes or proteins of an ethanol pathway, exogenous expression of all enzyme or proteins in the pathway can be included, although it is understood that all enzymes or proteins of a pathway can be expressed even if the host contains at least one of the pathway enzymes or proteins. For example, exogenous expression of all enzymes or proteins in a pathway for production of ethanol can be included in *S. cerevisiae* to enhance the production of ethanol from xylose, although *S. cerevisiae* has endogenous expression for all enzymes of the ethanol biosynthesis pathway from xylose except a xylose transporter.

[00193] Given the teachings and guidance provided herein, those skilled in the art will understand that the number of encoding nucleic acids to introduce in an expressible form will, at least, parallel the pathway deficiencies of the selected host microbial organism. Therefore, a non-naturally occurring microbial organism of the invention can have one, two, three, four, five, six, seven or eight up to all nucleic acids encoding the enzymes or proteins constituting a biosynthetic pathway. In some embodiments, the non-naturally occurring microbial organisms also can include other genetic modifications that facilitate or optimize biosynthesis of a particular compound or that confer other useful functions onto the host

microbial organism. One such other functionality can include, for example, augmentation of the synthesis of one or more of the pathway precursors for a particular compound.

[00194] Generally, a host microbial organism is selected such that it produces the desired product or the precursor of a desired product, either as a naturally produced molecule or as an engineered product that either provides *de novo* production of a desired precursor or increased production of a precursor naturally produced by the host microbial organism. For example, ethanol is produced naturally in a host organism such as *E. coli*. A host organism can be engineered to increase production of a precursor, as disclosed herein. In addition, a microbial organism that has been engineered to produce a desired precursor can be used as a host organism and further engineered to express enzymes or proteins of a particular biosynthesis pathway.

[00195] In some embodiments, a non-naturally occurring microbial organism provided herein is generated from a host that contains the enzymatic capability to synthesize compounds such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol from xylose. In this specific embodiment it can be useful to increase the synthesis or accumulation of the desired product to, for example, drive the biosynthesis pathway reactions toward the production of the desired product. Increased synthesis or accumulation can be accomplished by, for example, overexpression of nucleic acids encoding one or more of the biosynthesis pathway enzymes or proteins for producing compounds such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol from xylose. Overexpression of the enzyme or enzymes and/or protein or proteins of the biosynthesis pathways of desired pathway can occur, for example, through exogenous expression of the endogenous gene or genes, or through exogenous expression of the heterologous gene or genes. Therefore, the microbial organisms with enhanced xylose uptake as provided herein can be readily modified for producing a desired compound, for example, through overexpression of one, two, three, four, five, and up to all nucleic acids encoding the biosynthetic pathway enzymes or proteins for the desired product. In addition, a non-naturally occurring organism can be generated by mutagenesis of an endogenous gene that results in an increase in activity of an enzyme in the biosynthetic pathway.

[00196] In particularly useful embodiments, exogenous expression of the encoding nucleic acids is employed. Exogenous expression confers the ability to custom tailor the expression

and/or regulatory elements to the host and application to achieve a desired expression level that is controlled by the user. However, endogenous expression also can be utilized in other embodiments such as by removing a negative regulatory effector or induction of the gene's promoter when linked to an inducible promoter or other regulatory element. Thus, an
5 endogenous gene having a naturally occurring inducible promoter can be up-regulated by providing the appropriate inducing agent, or the regulatory region of an endogenous gene can be engineered to incorporate an inducible regulatory element, thereby allowing the regulation of increased expression of an endogenous gene at a desired time. Similarly, an inducible promoter can be included as a regulatory element for an exogenous gene introduced into a
10 non-naturally occurring microbial organism.

[00197] It is understood that any of the one or more exogenous nucleic acids can be introduced into a microbial organism to produce a non-naturally occurring microbial organism with increased production of a desired product, such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-
15 methyl-butanol. The nucleic acids can be introduced so as to confer, for example, a biosynthetic pathway to produce ethanol from xylose onto the microbial organism. Alternatively, encoding nucleic acids can be introduced to produce an intermediate microbial organism having the biosynthetic capability to catalyze some of the required reactions to confer biosynthetic capability. For example, a non-naturally occurring microbial organism
20 having a biosynthetic pathway can comprise at least two exogenous nucleic acids encoding desired enzymes or proteins. Thus, it is understood that any combination of two or more enzymes or proteins of a biosynthetic pathway can be included in a non-naturally occurring microbial organism of the invention. Similarly, it is understood that any combination of three or more enzymes or proteins of a biosynthetic pathway can be included in a non-naturally
25 occurring microbial organism of the invention so long as the combination of enzymes and/or proteins of the desired biosynthetic pathway results in production of the corresponding desired product. Similarly, any combination of four or more enzymes or proteins of a biosynthetic pathway as disclosed herein can be included in a non-naturally occurring microbial organism of the invention, as desired, so long as the combination of enzymes
30 and/or proteins of the desired biosynthetic pathway results in production of the corresponding desired product.

[00198] In addition to the biosynthesis of a desired product as described herein, the non-naturally occurring microbial organisms and methods provided herein also can be utilized in various combinations with each other and/or with other microbial organisms and methods well known in the art to achieve product biosynthesis by other routes. For example, one alternative to produce ethanol other than use of the ethanol producers is through addition of another microbial organism capable of converting an ethanol pathway intermediate to ethanol. One such procedure includes, for example, the fermentation of a microbial organism that produces an ethanol pathway intermediate. The ethanol pathway intermediate can then be used as a substrate for a second microbial organism that converts the ethanol pathway intermediate to ethanol. The ethanol pathway intermediate can be added directly to another culture of the second organism or the original culture of the ethanol pathway intermediate producers can be depleted of these microbial organisms by, for example, cell separation, and then subsequent addition of the second organism to the fermentation broth can be utilized to produce the final product without intermediate purification steps. Although ethanol is used as an example here, the same approach can be used for production of other desired products such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol.

[00199] In other embodiments, the non-naturally occurring microbial organisms and methods provided herein can be assembled in a wide variety of subpathways to achieve biosynthesis of a desired product. In these embodiments, biosynthetic pathways for a desired product of the invention can be segregated into different microbial organisms, and the different microbial organisms can be co-cultured to produce the final product. In such a biosynthetic scheme, the product of one microbial organism is the substrate for a second microbial organism until the final product is synthesized. For example, the biosynthesis of a desired product can be accomplished by constructing a microbial organism that contains biosynthetic pathways for conversion of one pathway intermediate to another pathway intermediate or the product. Alternatively, a desired product also can be biosynthetically produced from microbial organisms through co-culture or co-fermentation using two organisms in the same vessel, where the first microbial organism produces an intermediate for the desired product and the second microbial organism converts the intermediate to the desired product. The desired product can be xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol.

[00200] Given the teachings and guidance provided herein, those skilled in the art will understand that a wide variety of combinations and permutations exist for the non-naturally occurring microbial organisms and methods provided herein, together with other microbial organisms, with the co-culture of other non-naturally occurring microbial organisms having
5 subpathways and with combinations of other chemical and/or biochemical procedures well known in the art to produce a desired product.

[00201] In some embodiments, the methods provided herein to produce a bioderived compound further include separated from other components in the culture using a variety of methods well known in the art. The bioderived compound can be xylitol, ethanol, n-butanol,
10 isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. Such separation methods include, for example, extraction procedures as well as methods that include continuous liquid-liquid extraction, pervaporation, membrane filtration, membrane separation, reverse osmosis, electrodialysis, distillation, crystallization, centrifugation, extractive filtration, ion exchange chromatography, size exclusion
15 chromatography, adsorption chromatography, ultrafiltration, activated charcoal adsorption, pH adjustment and precipitation, or a combination of one or more methods enumerated above. All of the above methods are well known in the art.

[00202] Any of the non-naturally occurring microbial organisms described herein can be cultured to produce and/or secrete the desired bioderived compound including such as xylitol,
20 ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. For example, the microbial organisms provided herein can be cultured for the biosynthetic production of a desired compound. Accordingly, in some embodiments, provided herein are culture media containing a desired bioderived compound described herein or intermediate thereof. In some aspects, the culture medium can also be
25 separated from the non-naturally occurring microbial organisms that produced the a desired bioderived compound or intermediate thereof. Methods for separating a microbial organism from culture medium are well known in the art. Exemplary methods include filtration, flocculation, precipitation, centrifugation, sedimentation, and the like.

[00203] For the production of the desired bioderived compound including such as xylitol,
30 ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol, the microbial organisms provided herein are cultured in a medium with carbon source and other essential nutrients. In some embodiments, the

microbial organisms provided herein are cultured in an aerobic culture medium. In some embodiments, the microbial organisms provided herein are cultured in a substantially anaerobic culture medium. As described herein, one exemplary growth condition for achieving biosynthesis of a desired product such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol includes anaerobic culture or fermentation conditions. In certain embodiments, the non-naturally occurring microbial organisms provided herein can be sustained, cultured or fermented under anaerobic or substantially anaerobic conditions. Briefly, an anaerobic condition refers to an environment devoid of oxygen. Substantially anaerobic conditions include, for example, a culture, batch fermentation or continuous fermentation such that the dissolved oxygen concentration in the medium remains between 0 and 10% of saturation. Substantially anaerobic conditions also includes growing or resting cells in liquid medium or on solid agar inside a sealed chamber maintained with an atmosphere of less than 1% oxygen. The percent of oxygen can be maintained by, for example, sparging the culture with an N_2/CO_2 mixture or other suitable non-oxygen gas or gases.

[00204] It is sometimes desirable and can be highly desirable to maintain anaerobic conditions in the fermenter to reduce the cost of the overall process. Such conditions can be obtained, for example, by first sparging the medium with nitrogen and then sealing the flasks with a septum and crimp-cap. For strains where growth is not observed anaerobically, microaerobic or substantially anaerobic conditions can be applied by perforating the septum with a small hole for limited aeration. Exemplary anaerobic conditions have been described previously and are well-known in the art. Exemplary aerobic and anaerobic conditions are described, for example, in United States publication 2009/0047719, filed August 10, 2007. Fermentations can be performed in a batch, fed-batch or continuous manner, as disclosed herein. Fermentations can also be conducted in two phases, if desired. The first phase can be aerobic to allow for high growth and therefore high productivity, followed by an anaerobic phase of high yields.

[00205] If desired, the pH of the medium can be maintained at a desired pH, in particular neutral pH, such as a pH of around 7 by addition of a base, such as NaOH or other bases, or acid, as needed to maintain the culture medium at a desirable pH. The growth rate can be determined by measuring optical density using a spectrophotometer (600 nm), and the glucose uptake rate by monitoring carbon source depletion over time.

[00206] The culture medium for the microbial organisms provided herein can include xylose, either as the sole source of carbon or in combination with one or more co-substrates described herein or known in the art. The culture medium can further include other supplements, such as yeast extract, and/or peptone. The culture medium can further include, for example, any other carbohydrate source which can supply a source of carbon to the non-naturally occurring microorganism. Such sources include, for example: other sugars such as cellobiose, hemicelluloses, glucose, arabinose, galactose, mannose, fructose, sucrose and starch; or glycerol. The source can be biomass hydrolysate. Thus, the culture medium can include xylose and the co-substrate glucose. The culture medium can include xylose and the co-substrate cellobiose. The culture medium can include xylose and the co-substrate hemicellulose. The culture medium can include xylose and the co-substrate galactose. The culture medium can include xylose and the co-substrate glycerol.

[00207] The culture medium can have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or higher amount of sugar (w/v). In some embodiments, the culture medium can have 2% sugar. In some embodiments, the culture medium can have 4% sugar. In some embodiments, the culture medium can have 10% sugar. In some embodiments, the culture medium can have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, or higher amount of xylose (w/v). The culture medium can have 1% xylose. The culture medium can have 2% xylose. The culture medium can have 3% xylose. The culture medium can have 4% xylose. The culture medium can have 5% xylose. The culture medium can have 6% xylose. The culture medium can have 7% xylose. The culture medium can have 8% xylose. The culture medium can have 9% xylose. The culture medium can have 10% xylose. The culture medium can have 11% xylose. The culture medium can have 12% xylose. The culture medium can have 12% xylose. The culture medium can have 13% xylose. The culture medium can have 14% xylose. The culture medium can have 15% xylose. The culture medium can have 16% xylose. The culture medium can have 17% xylose. The culture medium can have 18% xylose. The culture medium can have 19% xylose. The culture medium can have 20% xylose.

[00208] The culture medium can be a C5-rich medium, with a five carbon sugar (such as xylose) as the primary carbon source. The culture medium can also have a C6 sugar (six-carbon sugar). In some embodiments, the culture medium can have a C6 sugar as the primary

carbon source. In some embodiments, the C6 sugar is glucose. The culture can have both a C6 sugar and a C5 sugar as the carbon source can have the C6 sugar and the C5 sugar present at different ratios. In some embodiment, the ratio of the amount of C6 sugar to that of the C5 sugar (the C6:C5 ratio) in the culture medium is between about 10:1 and about 1:10. For
5 example, the C6:C5 ratio in the culture medium can be about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10. In some embodiments, the C6:C5 ratio in the culture medium is about 3:1. In some embodiments, the C6:C5 ratio in the culture medium is about 1:1. In some embodiments, the C6:C5 ratio in the culture medium is about 1:5. In some embodiments, the C6:C5 ratio in the culture medium is about 1:10. The C5
10 sugar can be xylose, and the C6 sugar can be glucose. In some embodiment, the ratio of the amount of glucose to that of xylose (the glucose:xylose ratio) in the culture medium is between about 10:1 and about 1:10. For example, the glucose:xylose ratio in the culture medium can be about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 or 1:10. In some embodiments, the glucose:xylose ratio in the culture medium is about
15 3:1. In some embodiments, the glucose:xylose ratio in the culture medium is about 1:1. In some embodiments, the glucose:xylose ratio in the culture medium is about 1:5. In some embodiments, the glucose:xylose ratio in the culture medium is about 1:10.

[00209] Other sources of carbohydrate include, for example, renewable feedstocks and biomass. Exemplary types of biomasses that can be used as feedstocks in the methods of the
20 invention include cellulosic biomass, hemicellulosic biomass and lignin feedstocks or portions of feedstocks. Such biomass feedstocks contain, for example, carbohydrate substrates useful as carbon sources such as xylose, glucose, arabinose, galactose, mannose, fructose and starch. Given the teachings and guidance provided herein, those skilled in the art will understand that renewable feedstocks and biomass other than those exemplified above
25 also can be used for culturing the microbial organisms of the invention for the production of the desired bioderived compound including such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol.

[00210] Accordingly, given the teachings and guidance provided herein, those skilled in
30 the art will understand that a non-naturally occurring microbial organism can be produced that secretes the biosynthesized compounds described herein when grown on xylose as a carbon source. Such compounds include, for example, xylitol, ethanol, n-butanol, isobutanol,

isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol and any of the intermediate metabolites thereof. All that is required is to engineer in one or more of the required enzyme or protein activities to achieve biosynthesis of the desired compound or intermediate including, for example, inclusion of some or all of the biosynthetic pathways for producing the desired product. Accordingly, provided herein is a non-naturally occurring microbial organism that produces and/or secretes a desired product such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol when grown on a carbohydrate or other carbon source and produces and/or secretes an intermediate metabolites shown in the biosynthesis pathway of the desired compound when grown on xylose and optionally other carbohydrate or carbon source.

[00211] The non-naturally occurring microbial organisms provided herein are constructed using methods well known in the art as exemplified herein to exogenously express at least one nucleic acid encoding a xylose transporter in sufficient amounts to enhance xylose uptake and increase the production of a desired product from xylose. It is understood that the microbial organisms provided herein are cultured under conditions sufficient to produce a desired product such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. Following the teachings and guidance provided herein, the non-naturally occurring microbial organisms provided herein can achieve biosynthesis of the desired product resulting in intracellular concentrations between about 0.1-200 mM or more. Generally, the intracellular concentration of the desired product between about 3-150 mM, particularly between about 5-125 mM and more particularly between about 8-100 mM, including about 10 mM, 20 mM, 50 mM, 80 mM, or more. Intracellular concentrations between and above each of these exemplary ranges also can be achieved from the non-naturally occurring microbial organisms provided herein.

[00212] In some embodiments, culture conditions include anaerobic or substantially anaerobic growth or maintenance conditions. Exemplary anaerobic conditions have been described previously and are well known in the art. Exemplary anaerobic conditions for fermentation processes are described herein and are described, for example, in U.S. publication 2009/0047719. Any of these conditions can be employed with the non-naturally occurring microbial organisms as well as other anaerobic conditions well known in the art.

Under such anaerobic or substantially anaerobic conditions, the producer strains can synthesize the desired product at intracellular concentrations of 5-10 mM or more as well as all other concentrations exemplified herein. It is understood that, even though the above description refers to intracellular concentrations, the producing microbial organisms can
5 produce the desired product intracellularly and/or secrete the product into the culture medium.

[00213] Exemplary fermentation processes include, but are not limited to, fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation; and continuous fermentation and continuous separation. In an exemplary fed-batch fermentation
10 protocol, the production organism is grown in a suitably sized bioreactor sparged with an appropriate gas. Under anaerobic conditions, the culture is sparged with an inert gas or combination of gases, for example, nitrogen, N_2/CO_2 mixture, argon, helium, and the like. As the cells grow and utilize the carbon source, additional carbon source(s) and/or other nutrients are fed into the bioreactor at a rate approximately balancing consumption of the
15 carbon source and/or nutrients. The temperature of the bioreactor is maintained at a desired temperature, generally in the range of 22-37 degrees C, but the temperature can be maintained at a higher or lower temperature depending on the the growth characteristics of the production organism and/or desired conditions for the fermentation process. Growth continues for a desired period of time to achieve desired characteristics of the culture in the
20 fermenter, for example, cell density, product concentration, and the like. In a fed-batch fermentation process, the time period for the fermentation is generally in the range of several hours to several days, for example, 8 to 24 hours, or 1, 2, 3, 4 or 5 days, or up to two weeks, depending on the desired culture conditions. The pH can be controlled or not, as desired, in which case a culture in which pH is not controlled will typically decrease to pH 3-6 by the
25 end of the run. In some embodiment, the initial pH can first decrease and then increase during the cultivation period. In one embodiment, the initial pH of the medium is around 6, and during the cultivation period, the pH decreased first to 5.5 and later increased to around 6.5. Upon completion of the cultivation period, the fermenter contents can be passed through a cell separation unit, for example, a centrifuge, filtration unit, and the like, to remove cells
30 and cell debris. In the case where the desired product is expressed intracellularly, the cells can be lysed or disrupted enzymatically or chemically prior to or after separation of cells from the fermentation broth, as desired, in order to release additional product. The fermentation broth can be transferred to a product separations unit. Isolation of product

occurs by standard separations procedures employed in the art to separate a desired product from dilute aqueous solutions. Such methods include, but are not limited to, liquid-liquid extraction using a water immiscible organic solvent (*e.g.*, toluene or other suitable solvents, including but not limited to diethyl ether, ethyl acetate, tetrahydrofuran (THF), methylene
5 chloride, chloroform, benzene, pentane, hexane, heptane, petroleum ether, methyl tertiary butyl ether (MTBE), dioxane, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and the like) to provide an organic solution of the product, if appropriate, standard distillation methods, and the like, depending on the chemical characteristics of the product of the fermentation process.

10 **[00214]** In an exemplary fully continuous fermentation protocol, the production organism is generally first grown up in batch mode in order to achieve a desired cell density. When the carbon source and/or other nutrients are exhausted, feed medium of the same composition is supplied continuously at a desired rate, usually with relatively high sugar concentration, and fermentation liquid is withdrawn at the same rate. Under such conditions, the product
15 concentration in the bioreactor generally remains constant, as well as the cell density. The temperature of the fermenter is maintained at a desired temperature, as discussed above. During the continuous fermentation phase, it is generally desirable to maintain a suitable pH range for optimized production. The pH can be monitored and maintained using routine methods, including the addition of suitable acids or bases to maintain a desired pH range.
20 The bioreactor is operated continuously for extended periods of time, generally at least one week to several weeks and up to one month, or longer, as appropriate and desired. The fermentation liquid and/or culture is monitored periodically, including sampling up to every day, as desired, to assure consistency of product concentration and/or cell density. In continuous mode, fermenter contents are constantly removed as new feed medium is
25 supplied. The exit stream, containing cells, medium, and product, are generally subjected to a continuous product separations procedure, with or without removing cells and cell debris, as desired. Continuous separations methods employed in the art can be used to separate the product from dilute aqueous solutions, including but not limited to continuous liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene or other suitable solvents,
30 including but not limited to diethyl ether, ethyl acetate, tetrahydrofuran (THF), methylene chloride, chloroform, benzene, pentane, hexane, heptane, petroleum ether, methyl tertiary butyl ether (MTBE), dioxane, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and

the like), standard continuous distillation methods, and the like, or other methods well known in the art.

[00215] In addition to the culturing and fermentation conditions disclosed herein, growth condition for achieving biosynthesis of the desired product can include the addition of an
5 osmoprotectant to the culturing conditions. In certain embodiments, the non-naturally occurring microbial organisms provided herein can be sustained, cultured or fermented as described herein in the presence of an osmoprotectant. Briefly, an osmoprotectant refers to a compound that acts as an osmolyte and helps a microbial organism as described herein survive osmotic stress. Osmoprotectants include, but are not limited to, betaines, amino
10 acids, and the sugar trehalose. Non-limiting examples of such are glycine betaine, praline betaine, dimethylthetin, dimethylsulfonioproprionate, 3-dimethylsulfonio-2-methylproprionate, pipecolic acid, dimethylsulfonioacetate, choline, L-carnitine and ectoine. In one aspect, the osmoprotectant is glycine betaine. It is understood to one of ordinary skill in the art that the amount and type of osmoprotectant suitable for protecting a microbial
15 organism described herein from osmotic stress will depend on the microbial organism used. The amount of osmoprotectant in the culturing conditions can be, for example, no more than about 0.1 mM, no more than about 0.5 mM, no more than about 1.0 mM, no more than about 1.5 mM, no more than about 2.0 mM, no more than about 2.5 mM, no more than about 3.0 mM, no more than about 5.0 mM, no more than about 7.0 mM, no more than about 10mM,
20 no more than about 50mM, no more than about 100mM or no more than about 500mM.

[00216] The culture conditions can include, for example, liquid culture procedures as well as fermentation and other large scale culture procedures. As described herein, particularly useful yields of the biosynthetic products can be obtained under anaerobic or substantially anaerobic culture conditions.

25 **[00217]** The culture conditions described herein can be scaled up and grown continuously for manufacturing of a desired product. Exemplary growth procedures include, for example, fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation. All of these processes are well known in the art. Fermentation procedures are particularly useful for the biosynthetic
30 production of commercial quantities of a desired product. Generally, and as with non-continuous culture procedures, the continuous and/or near-continuous production includes culturing the microbial organisms provided herein in sufficient nutrients and medium to

sustain and/or nearly sustain growth in an exponential phase. Continuous culture under such conditions can include, for example, growth or culturing for 1 day, 2, 3, 4, 5, 6 or 7 days or more. Additionally, continuous culture can include longer time periods of 1 week, 2, 3, 4 or 5 or more weeks and up to several months. Alternatively, organisms of the invention can be cultured for hours, if suitable for a particular application. It is to be understood that the continuous and/or near-continuous culture conditions also can include all time intervals in between these exemplary periods. It is further understood that the time of culturing the microbial organism provided herein is for a sufficient period of time to produce a sufficient amount of product for a desired purpose.

[00218] Fermentation procedures are well known in the art. Briefly, fermentation for the biosynthetic production of a desired product can be utilized in, for example, fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation. Examples of batch and continuous fermentation procedures are well known in the art.

[00219] In addition to the above fermentation procedures using producer strains provided herein using continuous production of substantial quantities of a desire product, the bioderived product also can be, for example, simultaneously subjected to chemical synthesis and/or enzymatic procedures to convert the product to other compounds, or the bioderived product can be separated from the fermentation culture and sequentially subjected to chemical and/or enzymatic conversion to convert the product to other compounds, if desired.

[00220] To generate better producers, metabolic modeling can be utilized to optimize growth conditions. Modeling can also be used to design gene knockouts that additionally optimize utilization of the pathway (see, for example, U.S. patent publications US 2002/0012939, US 2003/0224363, US 2004/0029149, US 2004/0072723, US 2003/0059792, US 2002/0168654 and US 2004/0009466, and U.S. Patent No. 7,127,379). Modeling analysis allows reliable predictions of the effects on cell growth of shifting the metabolism towards more efficient production of a desired product.

[00221] Provided herein are also compositions having a bioderived compound produced by the microbial organisms described herein, and an additional component. The component other than the bioderived product can be a cellular portion, for example, a trace amount of a cellular portion of the culture medium, or can be fermentation broth or culture medium or a

purified or partially purified fraction thereof produced in the presence of, a non-naturally occurring microbial organism provided herein having a xylose transporter. The composition can have, for example, a reduced level of a byproduct when produced by the microbial organism disclosed herein. The composition can have, for example, one or more bioderived compound such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol, and a cell lysate or culture supernatant of a microbial organism provided herein. The additional component can be a byproduct, or an impurity, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof. The byproduct can be glycerol. The byproduct can be acetaldehyde. The byproduct can be glyceraldehyde. The byproduct can be acetate. The impurity can be glycerol. The impurity can be acetaldehyde. The impurity can be glyceraldehyde. The impurity can be acetate.

[00222] In some embodiments, the carbon feedstock and other cellular uptake sources such as phosphate, ammonia, sulfate, chloride and other halogens can be chosen to alter the isotopic distribution of the atoms present in the bioderived compound produced by microbial organisms provided herein. The various carbon feedstock and other uptake sources enumerated above will be referred to herein, collectively, as "uptake sources." Uptake sources can provide isotopic enrichment for any atom present in the bioderived compound produced by microbial organisms provided herein, or in the byproducts or impurities. Isotopic enrichment can be achieved for any target atom including, for example, carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, chloride or other halogens.

[00223] In some embodiments, the uptake sources can be selected to alter the carbon-12, carbon-13, and carbon-14 ratios. In some embodiments, the uptake sources can be selected to alter the oxygen-16, oxygen-17, and oxygen-18 ratios. In some embodiments, the uptake sources can be selected to alter the hydrogen, deuterium, and tritium ratios. In some embodiments, the uptake sources can be selected to alter the nitrogen-14 and nitrogen-15 ratios. In some embodiments, the uptake sources can be selected to alter the sulfur-32, sulfur-33, sulfur-34, and sulfur-35 ratios. In some embodiments, the uptake sources can be selected to alter the phosphorus-31, phosphorus-32, and phosphorus-33 ratios. In some embodiments, the uptake sources can be selected to alter the chlorine-35, chlorine-36, and chlorine-37 ratios.

[00224] In some embodiments, the isotopic ratio of a target atom can be varied to a desired ratio by selecting one or more uptake sources. An uptake source can be derived from a natural source, as found in nature, or from a man-made source, and one skilled in the art can select a natural source, a man-made source, or a combination thereof, to achieve a desired isotopic ratio of a target atom. An example of a man-made uptake source includes, for example, an uptake source that is at least partially derived from a chemical synthetic reaction. Such isotopically enriched uptake sources can be purchased commercially or prepared in the laboratory and/or optionally mixed with a natural source of the uptake source to achieve a desired isotopic ratio. In some embodiments, a target atom isotopic ratio of an uptake source can be achieved by selecting a desired origin of the uptake source as found in nature. For example, as discussed herein, a natural source can be a biobased derived from or synthesized by a biological organism or a source such as petroleum-based products or the atmosphere. In some such embodiments, a source of carbon, for example, can be selected from a fossil fuel-derived carbon source, which can be relatively depleted of carbon-14, or an environmental or atmospheric carbon source, such as CO_2, which can possess a larger amount of carbon-14 than its petroleum-derived counterpart.

[00225] The unstable carbon isotope carbon-14 or radiocarbon makes up for roughly 1 in 10^{12} carbon atoms in the earth's atmosphere and has a half-life of about 5700 years. The stock of carbon is replenished in the upper atmosphere by a nuclear reaction involving cosmic rays and ordinary nitrogen (^{14}N). Fossil fuels contain no carbon-14, as it decayed long ago. Burning of fossil fuels lowers the atmospheric carbon-14 fraction, the so-called “Suess effect”.

[00226] Methods of determining the isotopic ratios of atoms in a compound are well known to those skilled in the art. Isotopic enrichment is readily assessed by mass spectrometry using techniques known in the art such as accelerated mass spectrometry (AMS), Stable Isotope Ratio Mass Spectrometry (SIRMS) and Site-Specific Natural Isotopic Fractionation by Nuclear Magnetic Resonance (SNIF-NMR). Such mass spectral techniques can be integrated with separation techniques such as liquid chromatography (LC), high performance liquid chromatography (HPLC) and/or gas chromatography, and the like.

[00227] In the case of carbon, ASTM D6866 was developed in the United States as a standardized analytical method for determining the biobased content of solid, liquid, and gaseous samples using radiocarbon dating by the American Society for Testing and Materials

(ASTM) International. The standard is based on the use of radiocarbon dating for the determination of a product's biobased content. ASTM D6866 was first published in 2004, and the current active version of the standard is ASTM D6866-11 (effective April 1, 2011). Radiocarbon dating techniques are well known to those skilled in the art, including those
5 described herein.

[00228] The biobased content of a compound is estimated by the ratio of carbon-14 (^{14}C) to carbon-12 (^{12}C). Specifically, the Fraction Modern (Fm) is computed from the expression: Fm = (S-B)/(M-B), where B, S and M represent the $^{14}C/^{12}C$ ratios of the blank, the sample and the modern reference, respectively. Fraction Modern is a measurement of the deviation
10 of the $^{14}C/^{12}C$ ratio of a sample from "Modern." Modern is defined as 95% of the radiocarbon concentration (in AD 1950) of National Bureau of Standards (NBS) Oxalic Acid I (i.e., standard reference materials (SRM) 4990b) normalized to $\delta^{13}C_{VPDB}$=-19 per mil (Olsson, *The use of Oxalic acid as a Standard.* in, Radiocarbon Variations and Absolute Chronology, Nobel Symposium, 12th Proc., John Wiley & Sons, New York (1970)). Mass spectrometry
15 results, for example, measured by ASM, are calculated using the internationally agreed upon definition of 0.95 times the specific activity of NBS Oxalic Acid I (SRM 4990b) normalized to $\delta^{13}C_{VPDB}$=-19 per mil. This is equivalent to an absolute (AD 1950) $^{14}C/^{12}C$ ratio of $1.176 \pm 0.010 \times 10^{-12}$ (Karlen et al., *Arkiv Geofysik*, 4:465-471 (1968)). The standard calculations take into account the differential uptake of one isotope with respect to another, for example,
20 the preferential uptake in biological systems of ^{12}C over ^{13}C over ^{14}C, and these corrections are reflected as a Fm corrected for δ^{13}.

[00229] An oxalic acid standard (SRM 4990b or HOx 1) was made from a crop of 1955 sugar beet. Although there were 1000 lbs made, this oxalic acid standard is no longer commercially available. The Oxalic Acid II standard (HOx 2; N.I.S.T designation SRM 4990
25 C) was made from a crop of 1977 French beet molasses. In the early 1980's, a group of 12 laboratories measured the ratios of the two standards. The ratio of the activity of Oxalic acid II to 1 is 1.2933±0.001 (the weighted mean). The isotopic ratio of HOx II is -17.8 per mil. ASTM D6866-11 suggests use of the available Oxalic Acid II standard SRM 4990 C (Hox2) for the modern standard (see discussion of original vs. currently available oxalic acid
30 standards in Mann, *Radiocarbon*, 25(2):519-527 (1983)). A Fm = 0% represents the entire lack of carbon-14 atoms in a material, thus indicating a fossil (for example, petroleum based) carbon source. A Fm = 100%, after correction for the post-1950 injection of carbon-14 into

the atmosphere from nuclear bomb testing, indicates an entirely modern carbon source. As described herein, such a "modern" source includes biobased sources.

[00230] As described in ASTM D6866, the percent modern carbon (pMC) can be greater than 100% because of the continuing but diminishing effects of the 1950s nuclear testing programs, which resulted in a considerable enrichment of carbon-14 in the atmosphere as described in ASTM D6866-11. Because all sample carbon-14 activities are referenced to a "pre-bomb" standard, and because nearly all new biobased products are produced in a post-bomb environment, all pMC values (after correction for isotopic fraction) must be multiplied by 0.95 (as of 2010) to better reflect the true biobased content of the sample. A biobased content that is greater than 103% suggests that either an analytical error has occurred, or that the source of biobased carbon is more than several years old.

[00231] ASTM D6866 quantifies the biobased content relative to the material's total organic content and does not consider the inorganic carbon and other non-carbon containing substances present. For example, a product that is 50% starch-based material and 50% water would be considered to have a Biobased Content = 100% (50% organic content that is 100% biobased) based on ASTM D6866. In another example, a product that is 50% starch-based material, 25% petroleum-based, and 25% water would have a Biobased Content = 66.7% (75% organic content but only 50% of the product is biobased). In another example, a product that is 50% organic carbon and is a petroleum-based product would be considered to have a Biobased Content = 0% (50% organic carbon but from fossil sources). Thus, based on the well known methods and known standards for determining the biobased content of a compound or material, one skilled in the art can readily determine the biobased content and/or prepared downstream products that utilize of the invention having a desired biobased content.

[00232] Applications of carbon-14 dating techniques to quantify bio-based content of materials are known in the art (Currie et al., *Nuclear Instruments and Methods in Physics Research B,* 172:281-287 (2000)). For example, carbon-14 dating has been used to quantify bio-based content in terephthalate-containing materials (Colonna et al., *Green Chemistry*, 13:2543-2548 (2011)). Notably, polypropylene terephthalate (PPT) polymers derived from renewable 1,3-propanediol and petroleum-derived terephthalic acid resulted in Fm values near 30% (i.e., since 3/11 of the polymeric carbon derives from renewable 1,3-propanediol and 8/11 from the fossil end member terephthalic acid) (Currie et al., *supra*, 2000). In

contrast, polybutylene terephthalate polymer derived from both renewable 1,4-butanediol and renewable terephthalic acid resulted in bio-based content exceeding 90% (Colonna et al., *supra*, 2011).

[00233] Accordingly, in some embodiments, provided herein are bioderived compounds that have a carbon-12, carbon-13, and carbon-14 ratio that reflects an atmospheric carbon, also referred to as environmental carbon, uptake source. The bioderived compounds include such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. For example, in some aspects the bioderived compound can have an Fm value of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, 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% or as much as 100%. In some such embodiments, the uptake source is CO_2. In some embodiments, provided herein are bioderived compounds that have a carbon-12, carbon-13, and carbon-14 ratio that reflects petroleum-based carbon uptake source. In this aspect, the bioderived compounds provided herein can have an Fm value of less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 2% or less than 1%. In some embodiments, bioderived compounds provided herein can have a carbon-12, carbon-13, and carbon-14 ratio that is obtained by a combination of an atmospheric carbon uptake source with a petroleum-based uptake source. Using such a combination of uptake sources is one way by which the carbon-12, carbon-13, and carbon-14 ratio can be varied, and the respective ratios would reflect the proportions of the uptake sources.

[00234] Further, provided herein are also the products derived the bioderived compounds including such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol, wherein the bioderived compounds has a carbon-12, carbon-13, and carbon-14 isotope ratio of about the same value as the CO_2 that occurs in the environment. For example, in some aspects, provided herein are bioderived compounds having a carbon-12 versus carbon-13 versus carbon-14 isotope ratio of about the same value as the CO_2 that occurs in the environment, or any of the other ratios disclosed herein. It is understood, as disclosed herein, that a product can have a carbon-12

versus carbon-13 versus carbon-14 isotope ratio of about the same value as the CO_2 that occurs in the environment, or any of the ratios disclosed herein, wherein the product is generated from bioderived compounds as disclosed herein, wherein the bioderived product is chemically modified to generate a final product. Methods of chemically modifying a
5 bioderived product to generate a desired product are well known to those skilled in the art, as described herein.

[00235] Provided herein are also compositions having a bioderived compound produced by the microbial organisms described herein, and an additional component. The component other than the bioderived product can be a cellular portion, for example, a trace amount of a
10 cellular portion of the culture medium, or can be fermentation broth or culture medium or a purified or partially purified fraction thereof produced in the presence of, a non-naturally occurring microbial organism provided herein having a xylose transporter. The composition can have, for example, a reduced level of a byproduct when produced by the microbial organism disclosed herein. The composition can have, for example, one or more bioderived
15 compound such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol, and a cell lysate or culture supernatant of a microbial organism provided herein. The additional component can be a byproduct, or an impurity, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof. The byproduct can be glycerol. The byproduct can be acetaldehyde.
20 The byproduct can be glyceraldehyde. The byproduct can be acetate. The impurity can be glycerol. The impurity can be acetaldehyde. The impurity can be glyceraldehyde. The impurity can be acetate.

[00236] In some embodiments, the compositions provided herein can have a bioderived xylitol and an additional component. The additional component can be fermentation broth or
25 culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived xylitol. The additional component can be the cell lysate of the microbial organism
30 provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00237] In some embodiments, the compositions provided herein can have a bioderived ethanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived ethanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00238] In some embodiments, the compositions provided herein can have a bioderived n-butanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived n-butanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00239] In some embodiments, the compositions provided herein can have a bioderived isobutanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived isobutanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00240] In some embodiments, the compositions provided herein can have a bioderived isopropanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having

an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived isopropanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00241] In some embodiments, the compositions provided herein can have a bioderived arabitol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived arabitol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00242] In some embodiments, the compositions provided herein can have a bioderived ethyl acetate and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived ethyl acetate. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00243] In some embodiments, the compositions provided herein can have a bioderived phenyl-ethyl alcohol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived phenyl-ethyl alcohol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00244] In some embodiments, the compositions provided herein can have a bioderived 2-methyl-butanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived 2-methyl-butanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00245] In some embodiments, the compositions provided herein can have a bioderived 3-methyl-butanol and an additional component. The additional component can be fermentation broth or culture medium. The additional component can be the supernatant of fermentation broth or culture medium. The additional component can be a cellular portion of fermentation broth or culture medium. The additional component can be the microbial organisms having an exogenous nucleic acid encoding a xylose transporter as described herein used to produce the bioderived 3-methyl-butanol. The additional component can be the cell lysate of the microbial organism provided herein. The additional component can be a byproduct, such as glycerol, acetaldehyde, acetate, glyceraldehyde, or a combination thereof.

[00246] Provided herein are also biobased products having one or more bioderived compound produced by a non-naturally occurring microorganism described herein or produced using a method described herein. In some embodiments, provided herein are biobased products produced using a bioderived compound described herein, such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. Such manufacturing can include chemically reacting the bioderived compound (*e.g.* chemical conversion, chemical functionalization, chemical coupling, oxidation, reduction, polymerization, copolymerization and the like) into the final product. In some embodiments, provided herein are biobased products having a bioderived compound described herein, such as xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, or 3-methyl-butanol. In some embodiments, provided herein are biobased products having at least 2%, at least 3%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least

40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98% or 100% bioderived compound as disclosed herein.

[00247] It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention. Throughout this application various publications have been referenced. The disclosures of these publications in their entireties, including GenBank and GI number publications, are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

EXAMPLE I

Expression of Xylose Transporters in *H0 Metschnikowia sp.*

[00248] The yeast of *H0 Metschnikowia sp.* was grown in various culture media, and the expression of various xylose transporters was measured by transcriptome analysis. As referred to in the Table below, "FPKM" refers to "Fragments Per Kilobase of transcript per Million mapped reads"; all media included the standard formulation of 2% peptone and 1% yeast extract; final sugar concentrations were 2% total in all culture media; "High" and "Low" refer to the maximum and minimum FPKM values found in the three biological replicates tested.

Table: Transcriptome Analysis of Xylose Transporters in *H0 Metschnikowia sp.*

Gene	Glucose_FPKM			Xylose_FPKM		
	Average	Low	High	Average	Low	High
GXF1	3137.35	2408.44	3866.25	860.96	671.36	1050.56
XYT1	31.9043	21.8232	41.9855	1758.64	1354.27	2163.01
HXT5	3.52499	0	8.25012	322.102	247.985	396.218
GXS1/HGT12	1.78735	0	5.2273	42.4692	28.2335	56.705
HGT19/APS1	22.3382	13.4993	31.1771	2254.54	1738.24	2770.83
QUP2	25.0151	15.6231	34.4071	46.223	32.1743	60.2716
GXF2/GAL2	302.552	232.996	372.107	69.8126	50.0024	89.6228
Gene	**Galactose_FPKM**			**Cellobiose_FPKM**		
	Average	Low	High	Average	Low	High
GXF1	1865.95	1442.41	2289.49	321.954	248.525	395.383
XYT1	1309.91	1016.34	1603.48	900.511	701.542	1099.48
HXT5	386.68	298.795	474.564	614.885	477.428	752.342
GXS1/HGT12	150.957	112.586	189.328	119.261	88.3474	150.175
HGT19/APS1	2915.42	2230.39	3600.46	3723.89	2821.8	4625.99
QUP2	43.0144	29.7968	56.2321	51.0531	36.0561	66.0501
GXF2/GAL2	89.9721	65.6522	114.292	31.5998	19.9003	43.2992
Gene	**FP_media_FPKM**					
	Average	Low	High			
GXF1	2238.27	1726.85	2749.7			
XYT1	461.868	359.76	563.975			
HXT5	61.3177	42.1094	80.5259			
GXS1/HGT12	5.15064	0	10.7361			
HGT19/APS1	830.711	644.877	1016.54			
QUP2	39.3644	26.9207	51.8081			
GXF2/GAL2	40.5208	26.9909	54.0507			

EXAMPLE II

Engineering Enhanced Xylose Uptake in *H0 Metschnikowia sp.*

[00249] *H0 Metschnikowia sp.* was confirmed to have a robust xylose uptake and metabolism machinery, with the ability to consume and metabolize xylose as its sole carbon source. Xylose uptake is measured by growing H0 in known quantities of xylose and measuring the xylose remaining in the medium by high performance liquid chromatography. The quantity of xylose remaining is compared with a standard curve and the amount of said sugar in the inoculation medium. As shown in FIG. 1 and FIG. 2, efficient xylose transport was observed in wild type *H0 Metschnikowia sp.* The xylose uptake by the *H0 Metschnikowia sp.* was measured to be between 24 to 48 grams in 48 hours and 90 grams in 6 days (initial OD_{600}=0.2), which is significantly higher than the xylose uptake rate by yeasts known in the art. *See* Hector, *et al.*, *Applied microbiology and biotechnology* 80(4): 675-684 (2008) (reporting xylose uptake rate of 10-15 grams in 48 h by *S. cerevisiae* with initial OD_{600} =1.0 at aerobic conditions); Runquist, *et al.*, *Appl Microbiol Biotechnol* 82:123 (2009) (reporting xylose uptake rate of 4 grams in 48 h by yeast (TMB34XX) at anaerobic conditions); Apel *et al.*, *Scientific reports* 6 (2016)(reporting xylose uptake rate of 9 grams in 48 h by yeast at aerobic conditions).The xylose transport was further enhanced in *H0 Metschnikowia sp.* overexpressing Xyt1p, as also shown in FIG. 1 and FIG. 2. To overexpress Xyt1p, the H0 *XYT1* cassette was used which is comprised of H0 *TPI1* promoter driving *XYT1* and blasticidin expressed from the H0 *PGK1* promoter. The primers Y33 and Y33R amplified *XYT1* OFR from H0 genomic DNA with homology 30 and 31 bp of homology with the H0 *TPI1* promoter and H0 *RPL15A* terminator in vector DeBONO_E28.7. The *XYT1* amplicon was Gibson assembled into the EcoRI and SalI sites of DeBONO_E28.7. The resulting Xyt1p vector was linearized with NdeI. Primers Y41 and Y41R were used to amplify H0 *PGK1*pro-Blasticidin-H0*PGK1*terminator was amplified from DeBONO_E29. The resulting amplicon was recombined into the NdeI site of the digested H0TPI1pro-*XYL1*-RPL15Aterminator. The vector was electroporated into H0 at 1.5 kv and 25 uF and 200 ohm in a 0.1 cm cuvette. Electroporated cells were recovered in liquid YPD for 4 hours. Finally, cells were diluted to 30% with liquid YPD and 100 uL of cell mixture was plated on YPD agar containing 350 ug / mL of blasticidin. Individual colonies were picked after 48 h and restreaked onto YPD agar blasticidin medium. The blasticidin concentration required to select transgenic H0 was determined empirically.

EXAMPLE III

Engineering *S. cerevisiae* with Enhanced Xylose Uptake

[00250] *S. cerevisiae* does not have the functional machinery to efficiently utilize xylose as the carbon source. *S. cerevisiae* has a fully annotated genome, complete transcriptomic data and hundreds of tools developed for genetic and biochemical manipulation. The xylose transporters from the *H0 Metschnikowia sp.* were introduced into *S. cerevisiae* to increase xylose uptake and to synthesize bioderived product from renewable biomass. *S. cerevisiae* BY4742 was used as the genetic platform to heterologously over-express xylose transporter from the *H0 Metschnikowia sp.*

[00251] Genes encoding the following xylose transporters from the *H0 Metschnikowia sp.* were cloned Xyt1p, Gxf1p, ΔGxf1p (variant of Gxf1p with shorter N-terminus), Gxs1p/Hgt12p, and Hxt5p, and codon optimized for expression in BY4742. As shown in FIG.3, the expression of Xyt1p, the xylose transferred in 48 hours from the medium increased from about 10% in BY4742 to about 74% in BY4742 expressing Xyt1p.

[00252] Genes encoding Gxf1p, ΔGxf1p, Gxs1p/Hgt12p, and Hxt5p from *H0 Metschnikowia sp.* were synthesized and transformed into BY4742 for xylose transport testing by HPLC. Following the design for Xyt1p, genes encoding each of Gxf1p, ΔGxf1p, Gxs1p/Hgt12p, and Hxt5p was expressed from the *TEF* promoter and terminator derived from the plasmid pUG6. All open reading frames (ORFs) were selected for with nourseothricin.

[00253] Due to H0 CTG codon usage, all ORFs corresponding to H0 transporters were synthesized by ThermoFisher as double stranded "gene strings." H0 transporter ORFs were translated with the codon translation table provided above. The resulting amino acid sequence was converted back to DNA. The resulting DNA was entered into the ThermoFisher genestrings web interface. The web interface modified the nucleotide sequence such that the amino acid remained as desired but the nucleotides would be altered such to achieve nearly balanced ratio of adenine-thymidine to guanine-cytosine. The synthetic *XYT1* ORF was flanked by approximately 25 bp of homology with the *TEF* promoter at the 5' terminus and *TEF* terminator at the 3' terminus on pUG6 in order to facilitate Gibson assembly, respectively. The ORF was Gibson assembled into the pUG6, linearized with Y10, Y10R primers, deleting the G418resistance ORF. Using primers Y15 and Y15R, H0 *ADH1*promoter-*NAT*- H0 *PGK1* terminator was amplified from pZL29 and assembled into the *TEF* promoter- *XYT1*-*TEF* terminator plasmid. The complete plasmid containing *XYT1* is designated DeBONO_E35.3. E35.3 was used as base vector clone all of

GXF1, *ΔGXF1*, *GXS1/HGT12*, *HXT5*, *HGT19*. The DeBONO_E35.3 vector was amplified with Y53 and Y53R primers to linearize the vector and simultaneously, omitting *XYL1*, creating fragment Y53 (fY53). Each of the synthesized, codon optimized transporters were cloned into fY53 by Gibson assembly. The cassettes expressing transporters and NAT
5 resistance were linearized by PCR with primers Y16, Y16R or Y96i and Y95Ri for integration into dubious ORFs at loci YIL100W and YLR123C. The linearized transporters were integrated into said dubious loci using standard *Saccharomyces* electroporation or chemical transformation methods.

[00254] Transgenic yeasts were recovered with 100 ug/mL NAT in solid YPD medium.
10 *GXF2/GAL2* was synthesized as described above. *GXF2/GAL2* was cloned into a G418 resistance vector with general structure: *CCW12* promoter - *GXF2/GAL2* - H0 *DIT1* terminator. The promoter-terminator sequences were amplified from vector DeBONO_E54. This vector was linearized with primers Y83 and Y83R to yield fY83. The *GXF2/GAL2* genestring was Gibson assembled into fY83. The transporter cassette was linearized by PCR
15 with primers Y91i+Y93Ri for integration into the dubious ORF at locus YLR122C. The linearized cassette was transformed as described above and *GXF2/GAL2* transgenics were selected with 200 ug/mL of G418.

[00255] Relevant primer sequences used in this example are provided below.

SEQ ID NO:	Primer	Sequences
28	Primer Y10	GAAAAAACTGGTACCGTTTAATCAGTACTGACAATAAAAAGATTCTTGT
29	Primer Y10R	TAATTTCTCTTCGTATCCCATGGTTGTTTATGTTCGGATGTGATGTGAG
30	Primer Y15	ACGCCGCCATCCAGTGTCGAAAACGAGCTTTGTCTTGTAAAGAGTCTTCGGTCATTTTTA
31	Primer Y15R	GCGGCCGCATAGGCCACTAGTGGATCTGATCAATACATACAAGCATCTCACAATCACAAG
32	Primer Y33	TTTTTCACCCACAACAAATAATATCAAAAGATGGGTTACGAGGAAAAGCTTGTAGCGCCC

33	Primer Y33R	ACGAGAACACCCAGCTAAACGCGGTGCGCGTTAGACCGTGCCCGTCTTCTCGTCTGAAGA
34	Primer Y41	CAGAGCAGATTGTACTGAGAGTGCACCAGGCGCGCCCCATCCAGTGTCGAACCATCATTAAAAGAT
35	Primer Y41R	CTCCTTACGCATCTGTGCGGTATTTCACACCGCACTAGACAATACATACAAGCATCTCACAATCACAA
36	Primer Y53	TCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAAC
37	Primer Y53R	CTCACATCACATCCGAACATAAACAACC
38	Primer Y83	TATCCCGTCACTTCCACATTCG
39	Primer Y83R	TATTGATATAGTGTTTAAGCGAATGACAGAAG
40	Primer Y96i	ATAGAAAGCAAATAGTTATATAATTTTTCATGGACGTAGGTCTAGAGATCTGTTTAGCTTGC
41	Primer Y95Ri	AATGCAAAAGCGGCTCCTAAACAGAAATTCTTCAGTCAATACATACAAGCATCTCACAATCACAAG
42	Primer Y93Ri	TCGTCTATATCAAAACTGCATGTTTCTCTACGTCTAATTAAGGGTTCTCGAGAGCTCG
43	Primer Y91i	ACTTCAATAGACTTCAATAGAAAGCAAATAGTTATATGCCCTGAGGATGTATCTGG

EXAMPLE IV

Xylose Uptake by Wildtype and Ubiquitin-Deficient Xylose Transporters

[00256] The primary sequences of the *H0 Metschnikowia sp.* transporters described herein were examined for ubiquitination sites/residues with predictive tool 'UbiPred' (Tung and Ho, (2008), *BMC Bioinformatics*, 9, 310). Ubiquitin-deficient mutants of Hxt5p, Hgt19p, Xyt1p, Gxf1p and Gal2p, were engineered to replace all their cytoplasmic facing lysine ("K") residues that were identified as ubiquitination sites to Arginine ("R").

Table: Cytoplasmic facing lysine (“K”) residues (ATG/M is Residue No. 1))

Transporter	Residue No.	Transporter	Residue No.	Transporter	Residue No.
Hgt19p (SEQ ID NO: 44)	4	**Xyt1p (SEQ ID NO: 55)**	6	**Gal2p (SEQ ID NO: 46)**	23
	20		517		26
	30		539		35
	93				542
Hxt5p (SEQ ID NO: 45)	7	**Gxf1p (SEQ ID NO: 54)**	9		546
	10		24		
	29		538		
	43				
	58				

[00257] As shown in the xylose update assay, replacing K with R doubled and quadrupled xylose uptake for each of Δubq-Hxt5p and Δubq-HGT19p compared with the native transporters at 18 h and 64 h (FIGs. 4A and 4B). When all ubiquitination sites were removed from *H0 Metschnikowia sp.* Xyt1p and *H0 Metschnikowia sp.* Gal2p and expressed in host strain BY4742, the transporters were no longer functional. The recovered transgenic yeasts, producing ubiquitin free transporters were slow growing, requiring doubled growing time compared to yeasts expressing unmodified *H0 Metschnikowia sp.* Xyt1p and Gal2p. Ubiquitin free Gxf1p producing cells could not be recovered. These results indicate that ubiquitination sites, although inhibitory in Hxt5p and Hgt19p, are required in Xyt1p, Gxf1p and Gal2p for xylose transport and/or protein stability.

CLAIMS

We claim:

1. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid
5 sequence that is at least 89% identical to a *Metschnikowia* xylose transporter.

2. The non-naturally occurring microbial organism of claim 1, comprising exogenous nucleic acids encoding at least two, at least three, at least four, at least five, at least six, or at least seven xylose transporters.

3. The non-naturally occurring microbial organism of claim 1 or 2, wherein said xylose
10 transporter has an amino acid sequence that is at least 90%, at least 95%, at least 98%, or at least 99%, identical to said *Metschnikowia* xylose transporter.

4. The non-naturally occurring microbial organism of any one of claims 1 to 3, wherein said xylose transporter is a *Metschnikowia* xylose transporter.

5. The non-naturally occurring microbial organism of any one of claims 1 to 4, wherein
15 said *Metschnikowia* xylose transporter is selected from the group consisting of Xyt1p, Gxf1p, ΔGxf1p, Gxf2p/Gal2p, Gxs1p/Hgt12p, ΔGxs1p/ΔHgt12p, Hxt5p, Hxt2.6p, Qup2p, and Aps1p/Hgt19p.

6. The non-naturally occurring microbial organism of any one of claims 1 to 5, wherein said *Metschnikowia* xylose transporter is from a *H0 Metschnikowia sp.*

20 7. The non-naturally occurring microbial organism of any one of claims 1 to 5, wherein said *Metschnikowia* xylose transporter has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5 and 7-12.

8. The non-naturally occurring microbial organism of any one of claims 1 to 5, wherein said microbial organism comprising an exogenous nucleic acid is selected from the group
25 consisting of SEQ ID NOs: 13-17 and 19-27.

9. The non-naturally occurring microbial organism of claim 7, wherein said microbial organism has at least one exogenous nucleic acid encoding SEQ ID NO:1.

10. The non-naturally occurring microbial organism of claim 7, wherein said microbial organism has at least one exogenous nucleic acid encoding SEQ ID NO: 12.

11. The non-naturally occurring microbial organism of any one of claims 1 to 6, wherein said xylose transporter is ubiquitin-deficient.

5 12. The non-naturally occurring microbial organism of claim 11, wherein said *Metschnikowia* xylose transporter has amino acid substitutions at at least two lysine residues.

13. The non-naturally occurring microbial organism of claim 11, wherein said *Metschnikowia* xylose transporter has an amino acid sequence of SEQ ID NO: 44 or SEQ ID NO: 45.

10 14. The non-naturally occurring microbial organism of any one of claims 1 to 13, wherein said exogenous nucleic acid is codon-optimized to produce said xylose transporter in said microbial organism.

15. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid
15 sequence that is at least 74% identical to Xyt1p of a *Metschnikowia* species.

16. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 85% identical to Gxf1p of a *Metschnikowia* species.

17. A non-naturally occurring microbial organism comprising at least one exogenous
20 nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 89% identical to a ΔGxf1p of a *Metschnikowia* species.

18. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 71% identical to a Gxf2p/Gal2p of a *Metschnikowia* species.

25 19. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 71% identical to a Gxs1p/Hgt12p of a *Metschnikowia* species.

20. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 60% identical to a Hxt5p of a *Metschnikowia* species.

21. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 84% identical to a Hxt2.6p of a *Metschnikowia* species.

22. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 50% identical to a Qup2p of a *Metschnikowia* species.

23. A non-naturally occurring microbial organism comprising at least one exogenous nucleic acid encoding a xylose transporter, wherein said xylose transporter has an amino acid sequence that is at least 74% identical to a Aps1p/Hgt19p of a *Metschnikowia* species.

24. The non-naturally occurring microbial organism of any one of claims 15 to 23, wherein said *Metschnikowia* xylose transporter is from a *H0 Metschnikowia sp.*

25. The non-naturally occurring microbial organism of any one of claims 1 to 23, wherein said at least one exogenous nucleic acid is a heterologous nucleic acid.

26. The non-naturally occurring microbial organism of any one of claims 1 to 23, wherein said microbial organism is in an aerobic culture medium.

27. The non-naturally occurring microbial organism of any one of claims 1 to 23, wherein said microbial organism is in a substantially anaerobic culture medium.

28. The non-naturally occurring microbial organism of any one of claims 1 to 23, wherein the microbial organism is a species of bacteria or yeast.

29. The non-naturally occurring microbial organism of claim 28, wherein the microbial organism is a species of a yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Candida tropicalis*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Aspergillus terreus*, *Aspergillus niger*, *Chlamydomonas reinhardtii*, *Pichia pastoris*, *Rhizopus arrhizus*, *Rhizobus oryzae*, *Trichoderma reesei*, and *Yarrowia lipolytica*.

30. The non-naturally occurring microbial organism of claim 29, wherein the yeast is *Saccharomyces cerevisiae*.

31. The non-naturally occurring *Saccharomyces cerevisiae* of claim 29 having at least one exogenous nucleic acid encoding SEQ ID NO:1.

5 32. The non-naturally occurring *Saccharomyces cerevisiae* of claim 29 having at least one exogenous nucleic acid encoding SEQ ID NO:12.

33. The non-naturally occurring *Saccharomyces cerevisiae* of claim 29 having the exogenous nucleic acid of SEQ ID NO: 21.

34. The non-naturally occurring *Saccharomyces cerevisiae* of claim 29 having the
10 exogenous nucleic acid of SEQ ID NO: 27.

35. The non-naturally occurring microbial organism of claim 28, wherein the microbial organism is a species of a bacteria selected from the group consisting of *Escherichia coli*, *Klebsiella oxytoca*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Rhizobium etli*, *Bacillus subtilis*, *Corynebacterium*
15 *glutamicum*, *Gluconobacter oxydans*, *Zymomonas mobilis*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Streptomyces coelicolor*, *Clostridium acetobutylicum*, *Pseudomonas fluorescens*, and *Pseudomonas putida*.

36. The non-naturally occurring microbial organism of any one of claims 1 to 35, wherein said microbial organism further comprises a metabolic pathway capable of producing a
20 bioderived compound from xylose.

37. The non-naturally occurring microbial organism of claim 36, wherein said bioderived compound is selected from the group consisting of xylitol, ethanol, n-butanol, isobutanol, isopropanol, arabitol, ethyl acetate, phenyl-ethyl alcohol, 2-methyl-butanol, and 3-methyl-butanol.

25 38. A method of producing a bioderived compound comprising:

culturing the non-naturally occurring microbial organism of claim 36 or 37 under conditions and for a sufficient period of time to produce said bioderived compound, and

wherein said microbial organism comprises a pathway capable of producing the bioderived compound from xylose.

39. The method of claim 38, wherein the conditions comprise culturing the microbial organism in medium comprising xylose and a co-substrate selected from the group consisting of cellobiose, hemicellulose, glycerol, galactose, and glucose, or a combination thereof.

40. The method of claim 39, wherein the co-substrate is glucose.

5 41. The method of any one of claims 38 to 40, wherein the culturing comprises batch cultivation, fed-batch cultivation or continuous cultivation.

42. The method of any one of claims 38 to 41, wherein the method further comprises separating the bioderived compound from other components in the culture.

43. The method of claim 42, wherein the separating comprises extraction, continuous
10 liquid-liquid extraction, pervaporation, membrane filtration, membrane separation, reverse osmosis, electrodialysis, distillation, crystallization, centrifugation, extractive filtration, ion exchange chromatography, absorption chromatography, or ultrafiltration.

44. A bioderived compound produced by the method of any one of claims 38 to 43.

45. The bioderived compound of claim 44 comprising glycerol, acetaldehyde, acetate,
15 glyceraldehyde, or a combination thereof as impurities.

46. A composition comprising the bioderived xylitol of claim 44 or 45.

47. The composition of claim 46, wherein the composition is culture medium.

48. The composition of claim 47, wherein the composition is culture medium from which the microbial organism has been removed.

20

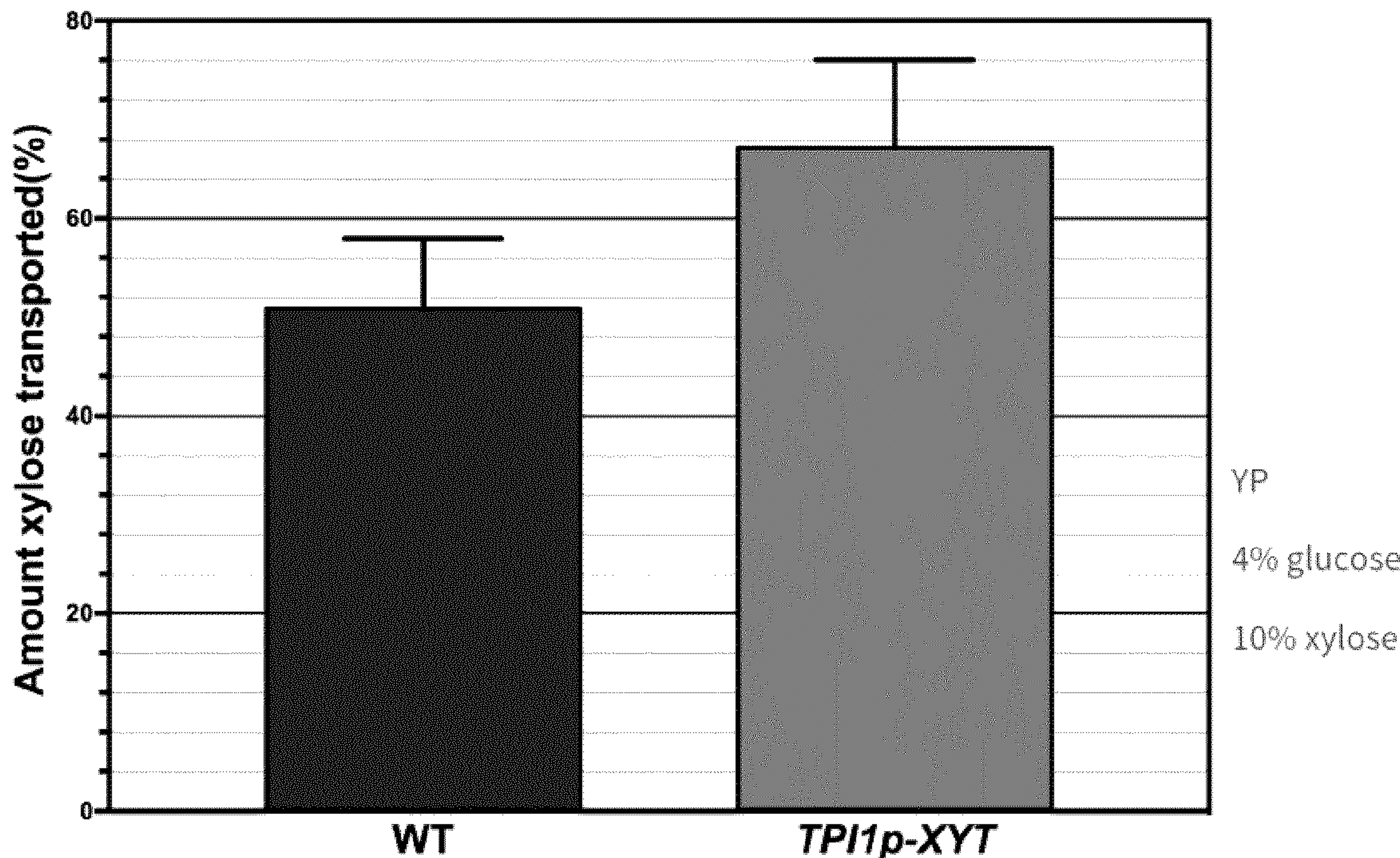

FIG. 1

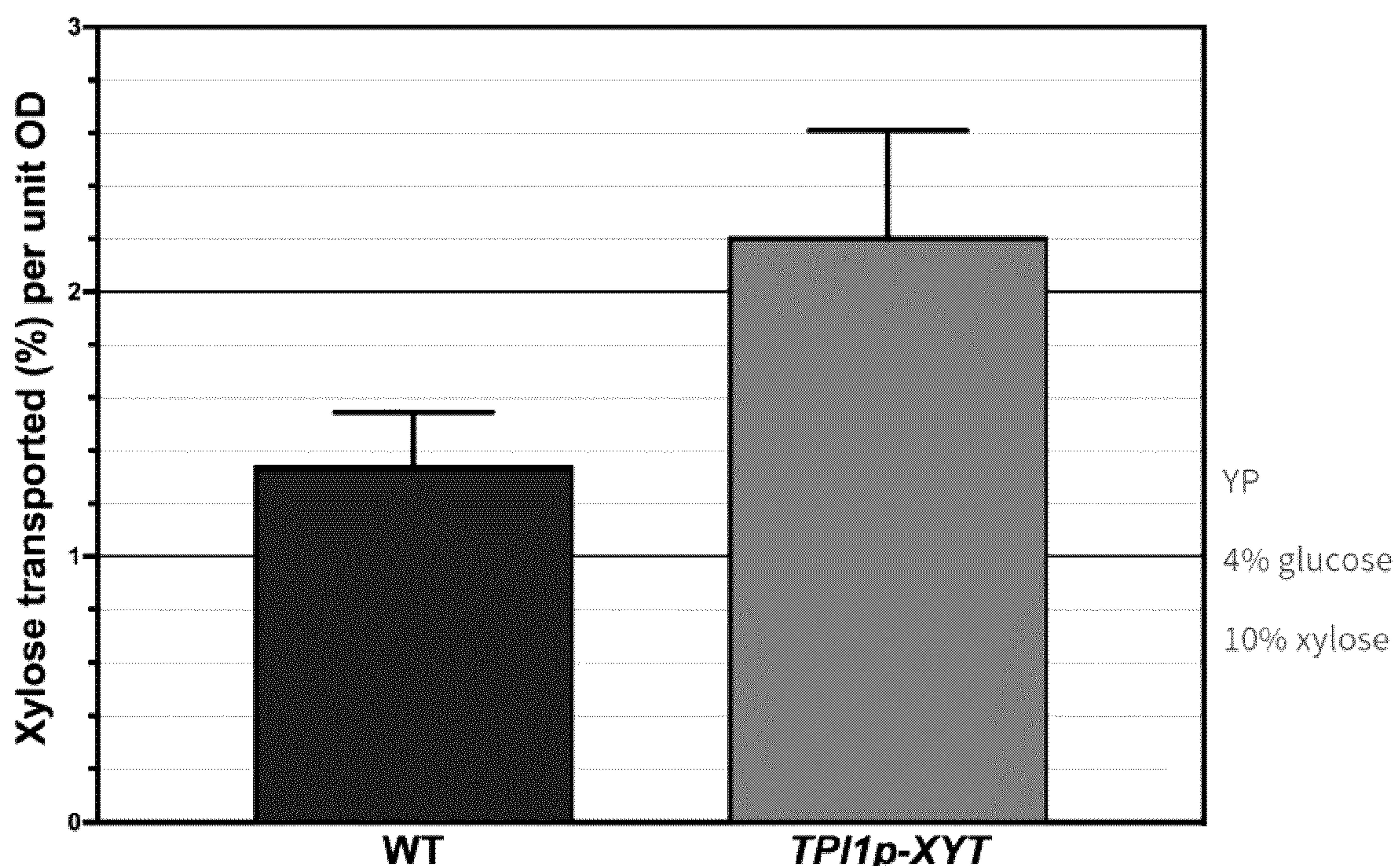

FIG. 2

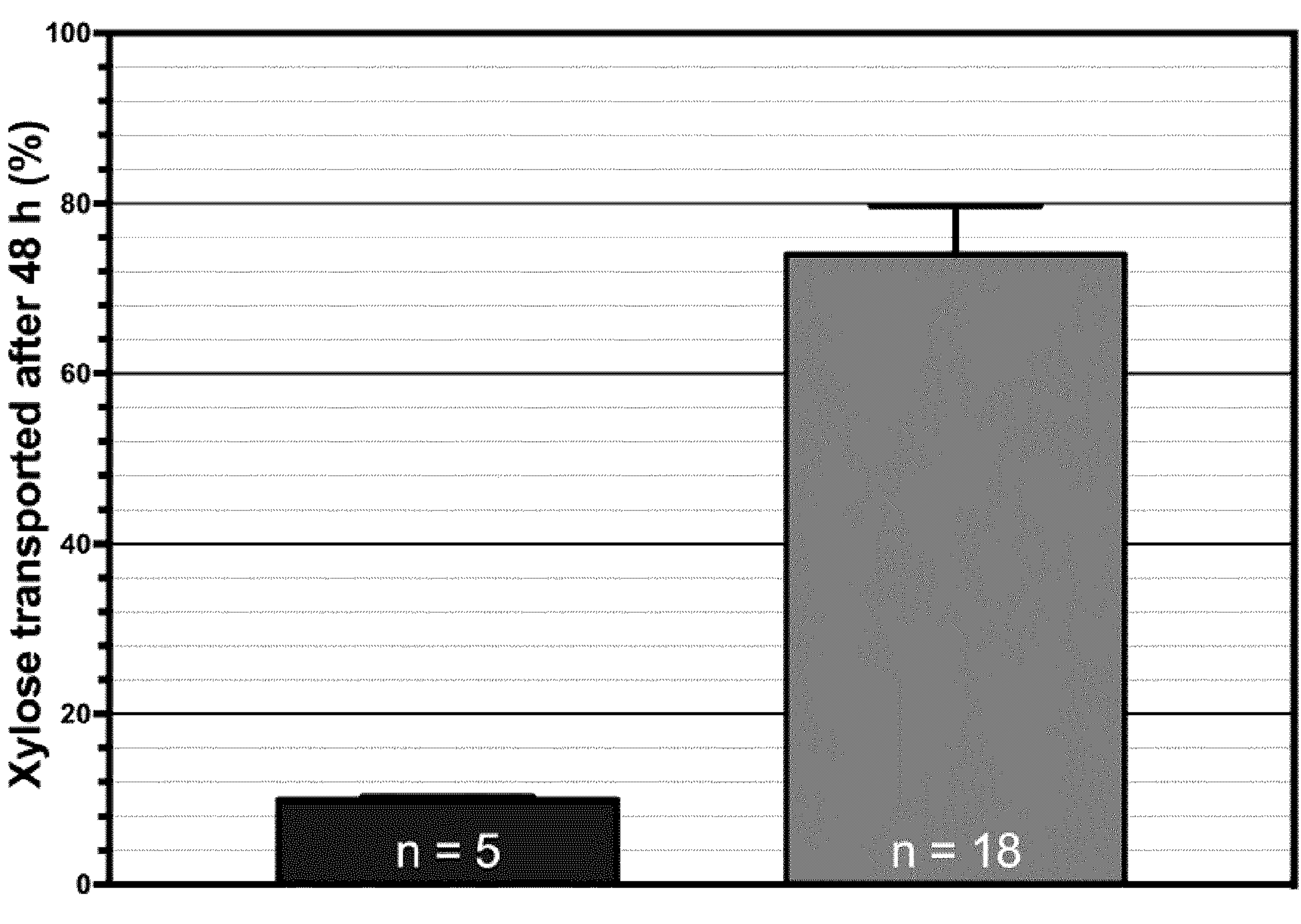

FIG. 3

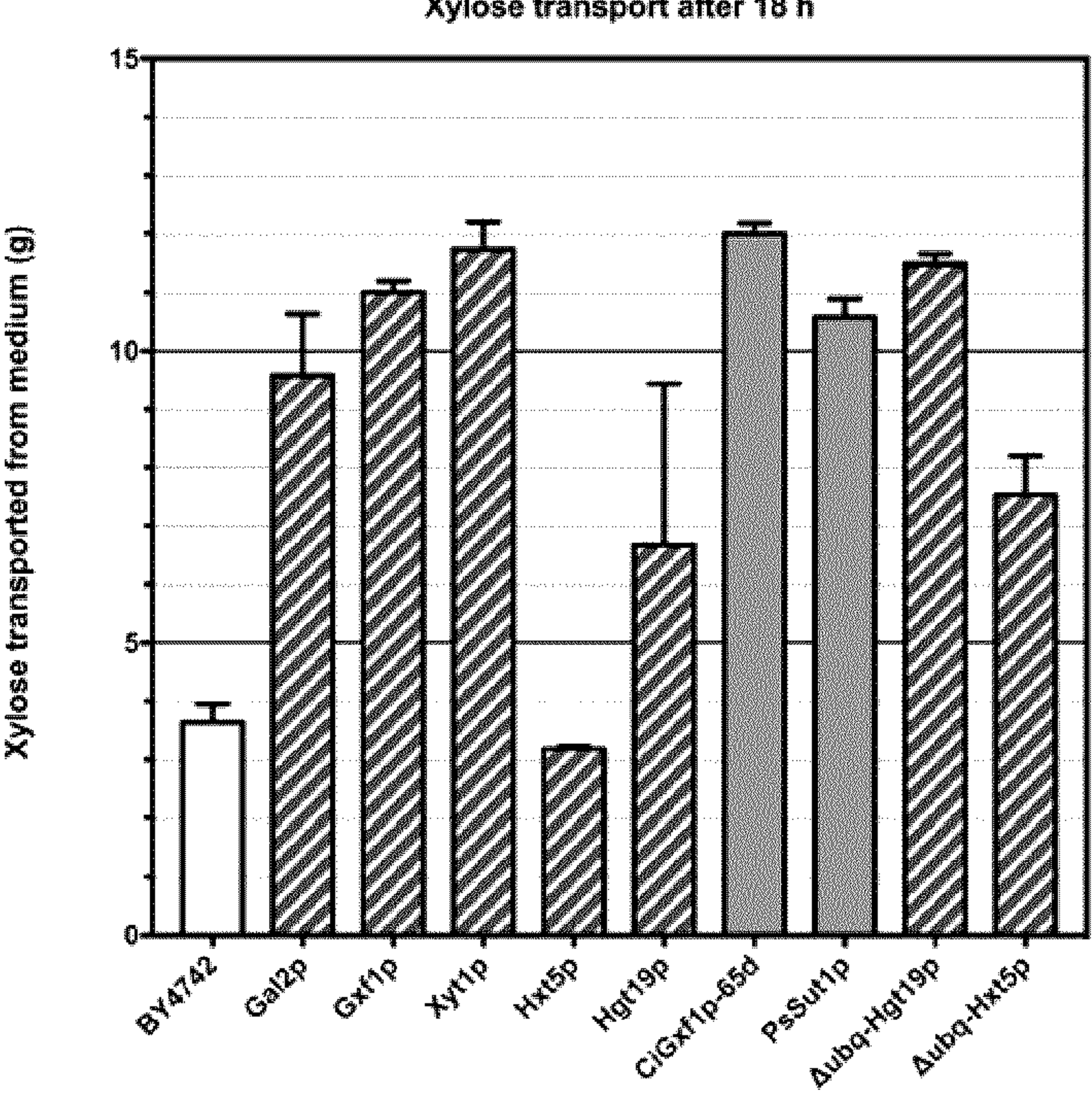

FIG. 4A

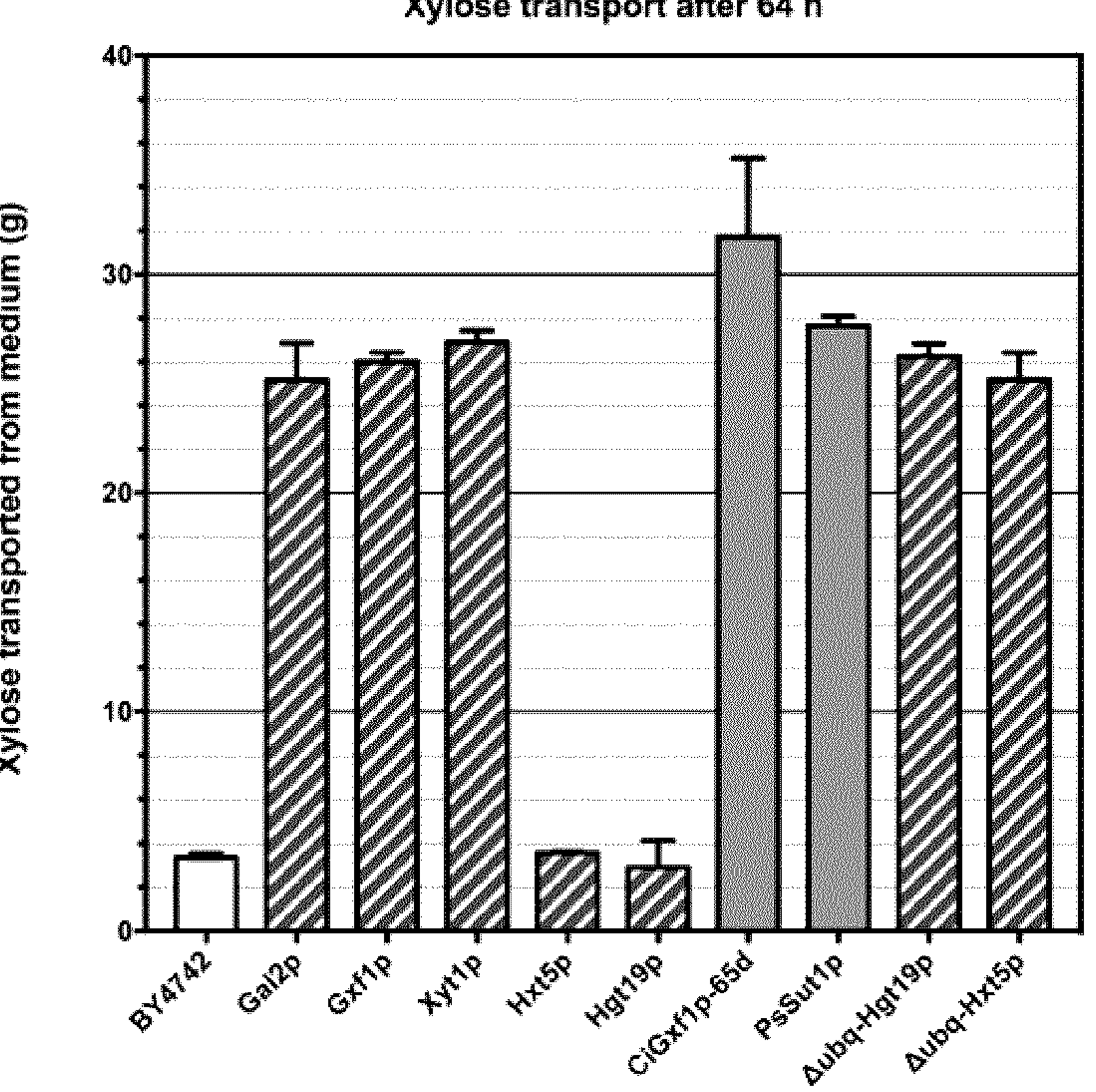

FIG. 4B

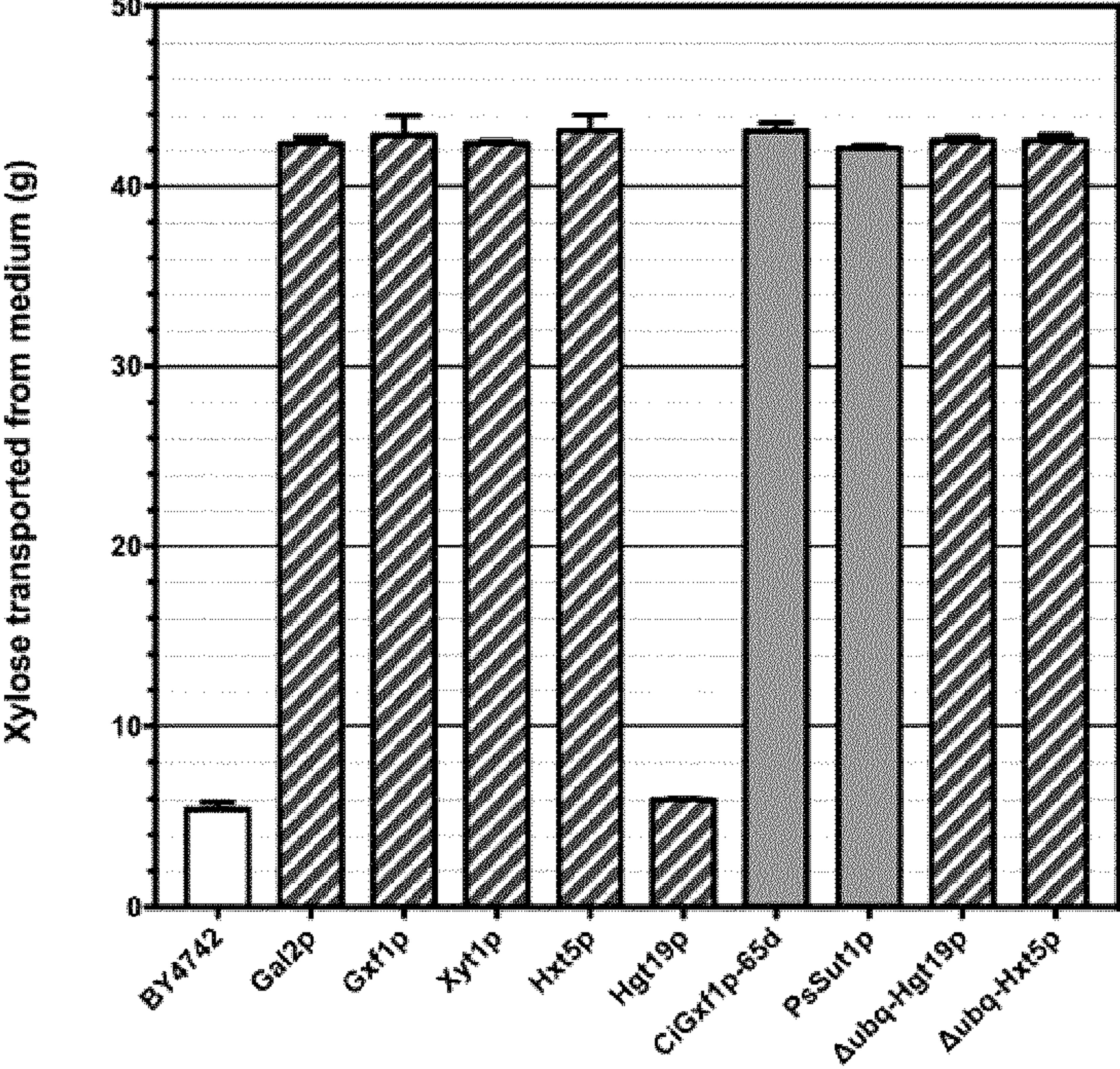

FIG. 4C

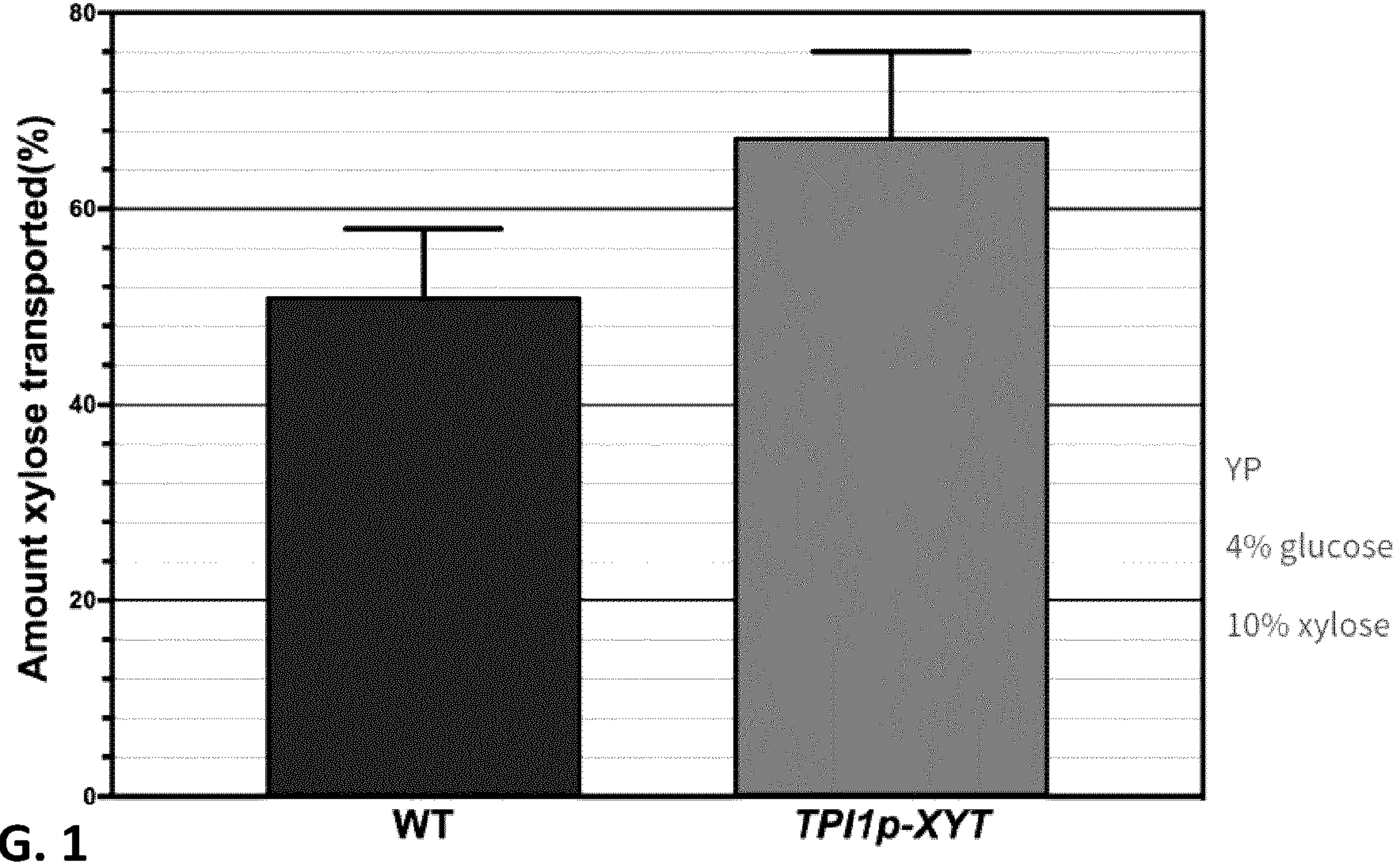

FIG. 1