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(54) Title: PENTOSE FERMENTATION BY A RECOMBINANT MICROORGANISM

(57) Abstract: The present invention provides methods and compositions suitable for use in the isomerization of xylose to xylulose, as well as methods and compositions suitable for use in the conversion of xylose to xylitol and xylulose, including nucleic acid constructs, recombinant fungal host cells, and related materials.

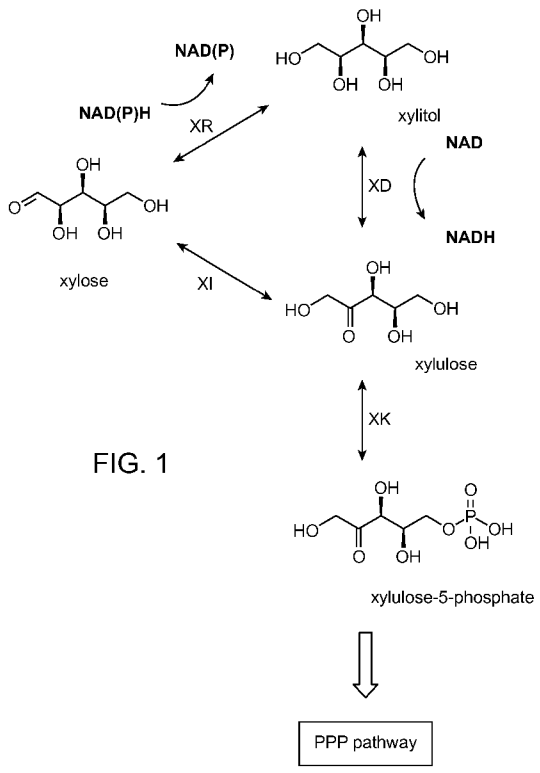

FIG. 1

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PENTOSE FERMENTATION BY A RECOMBINANT MICROORGANISM

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/728,398, filed November 20, 2012, the content of which is incorporated herein by reference in its entirety and for all purposes.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII FILE

[0002] The Sequence Listing written in file CX3-125WO1_ST25.TXT, created on November 5, 2013, 70,459 bytes, machine format IBM-PC, MS Windows operating system, is hereby incorporated by reference.

FIELD OF THE INVENTION

[0003] The present invention provides methods and compositions suitable for use in the isomerization of xylose to xylulose, as well as methods and compositions suitable for use in the conversion of xylose to xylitol and xylulose, including nucleic acid constructs, recombinant fungal host cells, and related materials.

BACKGROUND

[0004] Ethanol and ethanol fuel blends are widely used in Brazil and in the United States as a transportation fuel. Combustion of these fuels is believed to produce fewer of the harmful exhaust emissions (*e.g.*, hydrocarbons, nitrogen oxide, and volatile organic compounds (VOCs)) that are generated by the combustion of petroleum. Bioethanol is a particularly favored form of ethanol because the plant biomass from which it is produced utilizes sunlight, an energy source that is renewable. In the United States, ethanol is used in gasoline blends that are from 5% to 85% ethanol. Blends of up to 10% ethanol (E10) are approved for use in all gasoline vehicles in the U.S. and blends of up to 85% ethanol (E85) can be utilized in specially engineered flexible-fuel vehicles (FFV). The Brazilian government has mandated the use of ethanol-gasoline blends as a vehicle fuel, and the mandatory blend has been 25% ethanol (E25) since 2007.

[0005] Bioethanol is currently produced by the fermentation of hexose sugars that are obtained from carbon feedstocks. Currently, only the sugar from sugar cane and starch from feedstock such as corn can be economically converted. There is, however, much interest in using lignocellulosic feedstocks where the cellulose part of a plant is broken down to sugars and subsequently converted to ethanol. Lignocellulosic biomass is made up of cellulose, hemicelluloses, and lignin. Cellulose and hemicellulose can be hydrolyzed in a saccharification process to sugars that can be subsequently

converted to ethanol via fermentation. The major fermentable sugars from lignocelluloses are glucose and xylose. For economical ethanol yields, a strain that can effectively convert all the major sugars present in cellulosic feedstock would be highly desirable.

SUMMARY OF THE INVENTION

[0006] The present invention provides methods and compositions suitable for use in the isomerization of xylose to xylulose, as well as methods and compositions suitable for use in the conversion of xylose to xylitol and xylulose, including nucleic acid constructs, recombinant fungal host cells, and related materials.

[0007] In some embodiments, the present invention provides recombinant fungal host cells comprising at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, and/or at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and at least one polynucleotide encoding a xylulokinase. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic or prokaryotic enzymes. In some additional embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic enzymes. In some further embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are fungal enzymes. In some additional embodiments, the nucleic acid construct(s) further comprise(s) at least one genetic element that facilitates stable integration into a fungal host genome. In some embodiments, the genetic element facilitates integration into a fungal host genome by homologous recombination. In some additional embodiments, the genetic element comprises a prokaryotic or eukaryotic origin of replication and/or a centromeric maintenance sequence. In some embodiments, the origin of replication and/or centromeric maintenance sequence is a fungal sequence. In some additional embodiments, the fungal origin of replication is a yeast origin of replication. In yet some additional embodiments, at least one of the polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell. In some further embodiments, the promoter sequence is a fungal promoter sequence. In some embodiments, the fungal promoter sequence is a yeast promoter sequence. In some additional embodiments, the polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell. In some more embodiments, the polynucleotide sequence contains codons optimized for expression in a yeast cell. In some further embodiments, at least one polynucleotide is integrated into the host cell genome.

In yet some additional embodiments, the host cell has had one or more native genes deleted from its genome. In some embodiments, the deletion of the one or more native gene results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced production of by products, wherein comparison is made with respect to the corresponding host cell without the deletion(s). In some further embodiments, the host cell is altered to overexpress one or more polynucleotides. In some embodiments, the overexpression results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced product of by products, wherein comparison is made to the corresponding unaltered host cell. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at

least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino

acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and at least one polynucleotide encoding a xylulokinase. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase,

at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3; and SEQ ID NO:5. In some additional embodiments, the host cell is a yeast cell. In some further embodiments, the host cell is *Saccharomyces cerevisiae*.

[0008] The present invention also provides recombinant fungal host cells comprising at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase. In some embodiments, the xylose isomerase is a eukaryotic or prokaryotic enzyme, while in some alternative embodiments, the xylose isomerase is a eukaryotic enzyme. In some embodiments, the xylose isomerase is a *G. trabeum* xylose isomerase, an *Orpinomyces* xylose isomerase, a xylose isomerase obtained from a bovine rumen, a xylose isomerase obtained from a human gut, a *C. boidinii* xylose isomerase, *P. infestans* xylose isomerase, or *B. hominis* xylose isomerase. In some additional embodiments, at least one nucleic acid construct further comprises at least one genetic element that facilitates stable integration into a fungal host genome. In some embodiments, the genetic element facilitates integration into a fungal host genome by homologous recombination. In some additional embodiments, the genetic element comprises a prokaryotic or eukaryotic origin of replication and/or a centromeric plasmid maintenance sequence. In some embodiments, the origin of replication and/or centromeric plasmid maintenance sequence is a

fungal sequence. In some additional embodiments, the fungal origin of replication is a yeast origin of replication. In yet some additional embodiments, at least one of the polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell. In some further embodiments, the promoter sequence is a fungal promoter sequence. In some embodiments, the fungal promoter sequence is a yeast promoter sequence. In some additional embodiments, the polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell. In some more embodiments, the polynucleotide sequence contains codons optimized for expression in a yeast cell. In some further embodiments, at least one polynucleotide is integrated into the host cell genome. In yet some additional embodiments, the host cell has had one or more native genes deleted from its genome. In some embodiments, the deletion of one or more native gene results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced production of by products, wherein comparison is made with respect to the corresponding host cell without the deletion(s). In some further embodiments, the host cell is altered to overexpress one or more polynucleotides. In some embodiments, the overexpression results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced product of by products, wherein comparison is made to the corresponding unaltered host cell. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3; and SEQ ID NO:5. In some additional embodiments, the host cell is a yeast cell. In some further embodiments, the host cell is *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:8, 10, 12,

14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:7, 9, 11, 13, 15, 17, 19, 21, and/or 23. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at

least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at

least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and at least one polynucleotide encoding a xylulokinase. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least

100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a

polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the host cell is a yeast cell. In some further embodiments, the host cell is *Saccharomyces cerevisiae*.

[0009] The present invention also provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase. In some embodiments, the nucleic acid constructs comprise at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase. In some embodiments, the nucleic acid constructs comprise at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase. In some further embodiments, the present invention provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one polynucleotide sequence encoding at least one xylitol dehydrogenase, and/or at least one polynucleotide sequence encoding at least one xylulokinase. In some further embodiments, the present invention provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one xylose isomerase, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase, and/or at least one polynucleotide sequence encoding at least one xylulokinase. In some further embodiments, the present invention provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one xylose isomerase, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase, and at least one polynucleotide sequence encoding at

least one xylulokinase. In some embodiments, the xylose isomerase, and/or xylitol dehydrogenase, and/or xylulokinase are eukaryotic or prokaryotic enzymes. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase are eukaryotic or prokaryotic enzymes. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic or prokaryotic enzymes. In some embodiments, the xylose isomerase, and/or xylitol dehydrogenase, and/or xylulokinase are eukaryotic enzymes. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase are eukaryotic enzymes. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic enzymes. In some additional embodiments, the xylose isomerase, and/or xylitol dehydrogenase, and/or xylulokinase are fungal enzymes. In some additional embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase are fungal enzymes. In some additional embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are fungal enzymes. In some further embodiments, the xylose isomerase, and/or xylitol dehydrogenase, and/or xylulokinase are yeast enzymes. In some further embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase are yeast enzymes. In some further embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase are yeast enzymes. In some additional embodiments, at least one nucleic acid construct further comprises at least one genetic element that facilitates stable integration into a fungal host genome. In some embodiments, the genetic element facilitates integration into a fungal host genome by homologous recombination. In some additional embodiments, the genetic element comprises a prokaryotic or eukaryotic origin of replication and/or a centromeric plasmid maintenance sequence. In some embodiments, the origin of replication and/or centromeric plasmid maintenance sequence is a fungal sequence. In some additional embodiments, the fungal origin of replication is a yeast origin of replication. In yet some additional embodiments, at least one of the polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell. In some further embodiments, the promoter sequence is a fungal promoter sequence. In some embodiments, the fungal promoter sequence is a yeast promoter sequence. In some additional embodiments, the polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell. In some more embodiments, the polynucleotide sequence contains codons optimized for expression in a yeast cell. In some additional embodiments, at least one polynucleotide sequence encoding at least one xylose isomerase is operatively linked to a promoter sequence, and/or at least one polynucleotide sequence encoding at least one xylitol dehydrogenase is operatively linked to a promoter sequence, and/or at least one polynucleotide sequence encoding at least one xylulokinase is operatively linked to a promoter sequence, wherein the promoter sequences are functional in a fungal host cell. In some additional embodiments, at least one polynucleotide sequence encoding at least one xylose isomerase is operatively linked to a promoter sequence, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase is operatively linked to a promoter sequence, and/or at least one polynucleotide sequence encoding at least one xylulokinase is

operatively linked to a promoter sequence, wherein the promoter sequences are functional in a fungal host cell. In some additional embodiments, at least one polynucleotide sequence encoding at least one xylose isomerase is operatively linked to a promoter sequence, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase is operatively linked to a promoter sequence, and at least one polynucleotide sequence encoding at least one xylulokinase is operatively linked to a promoter sequence, wherein the promoter sequences are functional in a fungal host cell. In some embodiments, the promoter sequence(s) is/are fungal promoter sequence(s). In some embodiments, the fungal promoter sequence is a yeast promoter sequence. In some additional embodiments, the polynucleotide sequence is operatively linked to at least one transcription termination sequence that is functional in a fungal cell. In some further embodiments, the polynucleotide sequence contains codons optimized for expression in a yeast cell. In still some additional embodiments, the construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8,

10, 12, 14, 16, 18, 20, 22, and/or 24. In still some additional embodiments, the construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about

100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some further embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the

complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some other embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, at least one polynucleotide sequence encodes at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide comprises at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3, and SEQ ID NO:5. In some additional embodiments, any of the constructs described above further comprises a polynucleotide sequence encoding at least one xylose reductase.

[0010] The present invention also provides recombinant nucleic acid constructs comprising at least one polynucleotide sequence encoding at least one xylose isomerase. In some embodiments, the xylose isomerase is a eukaryotic or prokaryotic enzyme, while in some alternative embodiments, the xylose isomerase is a eukaryotic enzyme. In some embodiments, the xylose isomerase is a *G. trabeum* xylose isomerase, an *Orpinomyces* xylose isomerase, a xylose isomerase obtained from a bovine rumen, a xylose isomerase obtained from a human gut, a *C. boidinii* xylose isomerase, *P. infestans* xylose isomerase, or *B. hominis* xylose isomerase. In some additional embodiments, at least one nucleic acid construct further comprises at least one genetic element that facilitates stable integration into a fungal host genome. In some embodiments, the genetic element facilitates integration into a fungal host genome by homologous recombination. In some additional embodiments, the genetic element comprises a prokaryotic or eukaryotic origin of replication and/or a centromeric plasmid maintenance sequence. In some embodiments, the origin of replication and/or centromeric plasmid maintenance sequence is a fungal sequence. In some additional embodiments, the fungal origin of replication is a yeast origin of replication. In yet some additional embodiments, at least one of the polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell. In some further embodiments, the promoter sequence is a fungal promoter sequence. In some embodiments, the fungal promoter sequence is a yeast promoter sequence. In some additional embodiments, the polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell. In some more embodiments, the polynucleotide sequence contains codons optimized for expression in a yeast cell. In some further embodiments, at least one polynucleotide is integrated into the host cell genome. In yet some additional embodiments, the host cell has had one or more native genes deleted from its genome. In some embodiments, the deletion of one or more native gene results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose

reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced production of by products, wherein comparison is made with respect to the corresponding host cell without the deletion(s). In some further embodiments, the host cell is altered to overexpress one or more polynucleotides. In some embodiments, the overexpression results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced product of by products, wherein comparison is made to the corresponding unaltered host cell. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3; and SEQ ID NO:5. In some additional embodiments, the host cell is a yeast cell. In some further embodiments, the host cell is *Saccharomyces cerevisiae*. In some embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the

amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:7, 9, 11, 13, 15, 17, 19, 21, and/or 23. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about

85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at

least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, the recombinant fungal host cell comprises at least one nucleic acid construct, wherein the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase. In some embodiments, the construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some further embodiments, the nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some additional embodiments, at least one polynucleotide sequence

encodes at least one xylose isomerase, wherein the polynucleotide comprises at least one sequence selected from SEQ ID NOS:7, 9, 11, 13, 15, 17, 19, 21, and 23. In some further embodiments, the nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, at least one polynucleotide encoding a xylitol dehydrogenase, and at least one polynucleotide encoding a xylulokinase. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, and/or at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and/or at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the nucleic acid construct(s) comprise(s) at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. In some embodiments, the host cell is a yeast cell. In some further embodiments, the host cell is *Saccharomyces cerevisiae*.

[0011] The present invention also provides isolated polypeptide sequences comprising a xylose isomerase polypeptide, and/or xylitol dehydrogenase polypeptide, and/or xylulokinase polypeptide. The present invention also provides isolated polypeptide sequences comprising at least one xylose isomerase polypeptide, and/or at least one xylitol dehydrogenase polypeptide, and/or at least one xylulokinase polypeptide. The present invention also provides isolated polypeptide sequences

comprising at least one xylose isomerase polypeptide, at least one xylitol dehydrogenase polypeptide, and/or at least one xylulokinase polypeptide. In some embodiments, the xylose isomerase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and the xylulokinase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6. In some further embodiments, the xylose isomerase polypeptide comprises an amino acid sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polypeptide comprises an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and the xylulokinase polypeptide comprises an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some additional embodiments, the xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and the xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5. In some further embodiments, the xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least 70%, % 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and the xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5. In still some additional embodiments, the xylose isomerase polypeptide is encoded by a polynucleotide sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide is encoded by SEQ ID NO:3; and the xylulokinase is encoded by SEQ ID NO:5.

[0012] The present invention further provides isolated polynucleotide sequences comprising a xylose isomerase polynucleotide, xylitol dehydrogenase polypeptide, and xylulokinase polypeptide. In some embodiments, the isolated polynucleotide sequences comprise at least one xylose isomerase polynucleotide, and/or at least one xylitol dehydrogenase polypeptide, and/or at least one xylulokinase polypeptide. In some further embodiments, the isolated polynucleotide sequences comprise at least one xylose isomerase polynucleotide, at least one xylitol dehydrogenase polypeptide, and/or at least one xylulokinase polypeptide. In some other embodiments, the isolated polynucleotide sequences comprise at least one xylose isomerase polynucleotide, at least one xylitol dehydrogenase polypeptide, and at least one xylulokinase polypeptide. In some additional embodiments, the xylose isomerase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; and/or the xylitol dehydrogenase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and/or the xylulokinase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about

80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5. In some further embodiments, the xylose isomerase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and/or the xylulokinase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5. In still some additional embodiments, the xylose isomerase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about

83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and the xylulokinase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5. In some embodiments, the xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; and/or the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and/or the xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5. In some embodiments, the xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and/or the xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5. In some embodiments, the xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and the xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5. In some further embodiments, the xylose isomerase polynucleotide sequence is selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; and/or the xylitol dehydrogenase polynucleotide sequence is SEQ ID NO:3; and/or the xylulokinase polynucleotide sequence is SEQ ID NO:5. In some further embodiments, the xylose isomerase polynucleotide sequence is selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polynucleotide sequence is SEQ ID NO:3; and/or the xylulokinase polynucleotide sequence is SEQ ID NO:5. In some further embodiments, the xylose isomerase polynucleotide sequence is selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polynucleotide sequence is SEQ ID NO:3; and the xylulokinase polynucleotide sequence is SEQ ID NO:5. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having at least about 70%, at least about 71%, at least

about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and/or the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and/or the xylulokinase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and/or the xylulokinase polynucleotide encodes an amino acid

sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and the xylulokinase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6. In some further embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and/or the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and/or the xylulokinase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some further embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and/or the xylulokinase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some further embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and the xylulokinase

polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence selected from SEQ ID NOS:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and/or the xylitol dehydrogenase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:4; and/or the xylulokinase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:6. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence selected from SEQ ID NOS:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:4; and/or the xylulokinase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:6. In some additional embodiments, the xylose isomerase polynucleotide sequence encodes an amino acid sequence selected from SEQ ID NOS:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:4; and the xylulokinase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:6.

[0013] The present invention also provides methods for producing a fermentation product, comprising: providing a recombinant fungal host cell as provided herein; providing a fermentation medium; and contacting the fermentation medium with the recombinant fungal host cell under conditions suitable for generating the fermentation product. In some embodiments, the methods further comprise the step of recovering the fermentation product. In some additional embodiments, the fermenting step is carried out under conditions selected from anaerobic, microaerobic or aerobic conditions. In some further embodiments, the fermentation product is selected from an alcohol, a fatty alcohol, a fatty acid, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, succinic acid, an amino acid, 1,3-propanediol, ethylene, glycerol, and a β-lactam. In some still additional embodiments, the fermentation product is an alcohol selected from ethanol and butanol. In some embodiments, the fermentation product is ethanol. In some embodiments, the fermentation medium comprises product from a saccharification process. In some further embodiments, the fermentation medium comprises hemicellulosic feedstock.

[0014] The present invention also provides methods of producing at least one end product from at least one cellulosic substrate, comprising: providing at least one cellulosic substrate and at least one enzyme composition comprising at least one cellulase; contacting the cellulosic substrate with the enzyme composition under conditions whereby fermentable sugars are produced from the cellulosic substrate in a saccharification reaction; and contacting the fermentable sugars with a microorganism under fermentation conditions such that at least one end product is produced. In some embodiments,

the methods comprise simultaneous saccharification and fermentation reactions (SSF), while in some alternative embodiments, the methods comprise saccharification of the cellulosic substrate and the fermentation in separate reactions (SHF). In some further embodiments, the enzyme composition is produced simultaneously with the saccharification reaction and the fermentation. In some embodiments, the methods further comprise at least one adjunct composition in the saccharification reaction. In some additional embodiments, the adjunct composition is selected from at least one divalent metal cation, copper, gallic acid, and/or at least one surfactant. In some further embodiments, the methods are conducted at about pH 5.0, while in some alternative embodiments, the methods are conducted at about pH 6.0. In some further embodiments, the methods further comprise recovering at least one end product. In some embodiments, the end product comprises at least one fermentation end product. In some additional embodiments, the fermentation end product is selected from alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, fatty alcohols, butadiene, and beta-lactams. In still some other embodiments, the fermentation end product is at least one alcohol selected from ethanol and butanol. In some embodiments, the alcohol is ethanol. In some additional embodiments, the microorganism is a yeast. In some embodiments, the yeast is *Saccharomyces*. In some additional embodiments, the methods further comprise recovering at least one fermentation end product.

DESCRIPTION OF THE FIGURES

[0015] Figure 1 illustrates xylose conversion pathways. In yeast and filamentous fungi, D-xylose is initially reduced to xylitol by NAD(P)H-dependent xylose reductase ("XR"). Xylitol is subsequently oxidized to D-xylulose by NAD+-dependent xylitol dehydrogenase ("XDH" or "XD"). Xylulokinase ("XK") subsequently phosphorylates D-xylulose to produce D-xylulose 5-phosphate, which is then further metabolized through the pentose phosphate pathway ("PPP"). In bacteria, D-xylose is directly converted to D-xylulose by a xylose isomerase ("XI").

[0016] Figures 2A-C illustrate the metabolic pathways for converting D-xylulose-5-P to ethanol.

[0017] Figure 2A illustrates the pentose phosphate pathway (PPP). The substrates and products are shown. The enzymes are represented by numbers as follows: 6. Ribulose-5-phosphate 3-epimerase; 7. Transketolase (TKL1); 8. Transaldolase (TAL1); 9. Ribose-5-phosphate ketoisomerase (RKI1); 10. 6-phosphogluconate dehydrogenase (GND1); 11. 6-phosphogluconalactonase (SOL3); and 12. Glucose-6-phosphate-1-dehydrogenase (ZWF).

[0018] Figure 2B illustrates the pathway of glycolysis. The substrates and products are shown. The enzymes are represented by numbers as follows: 13. Hexokinase; 14. Phosphoglucose isomerase; 15. Phosphofructokinase; 16. Aldolase; 17. Triose phosphate isomerase; 18. Glyceraldehyde 3-phosphate dehydrogenase; 19. 3-Phosphoglycerate kinase; 20. Phosphoglyceromutase; 21. Enolase; and 22. Pyruvate kinase.

[0019] Figure 2C illustrates the metabolic pathway for converting pyruvate to ethanol. The substrates and products are shown. The enzymes are represented by numbers as follows: 23. Pyruvate decarboxylase; 24. Aldehyde dehydrogenase; and 25. Alcohol dehydrogenase.

[0020] Figure 3 provides a graph showing the fermentation results of strains comprising XI, XD and XK genes in different combinations. Fermentation performed in 96-well plates for 96 hours in YPD media supplemented with 30g/l xylose. Residual xylose, as well as produced ethanol and xylitol are shown. Numbers in boxes represent xylose consumed in comparison with a control strain comprising an empty plasmid (n=7, error bars ±SD).

[0021] Figure 4 provides a graph showing the fold improvement in xylose consumption under several fermentation conditions by different strains comprising the XI-XD-XK pathway relative to xylose consumption by the same strains comprising the XI gene only.

[0022] Figure 5 provides a graph showing the time course analysis of 25-ml fermentation of haploid strains comprising an empty plasmid (negative control), XI-XD-XK pathway or XI gene only.

[0023] Figure 6 provides a map of the XIDK integration construct.

[0024] Figure 7 provides a graph showing the residual xylose as measured after fermentation of NRRL Y1528-derived haploid strains comprising XI genes from diverse origins. Fermentation was performed in 96-well plates for 72 hours in YPD media supplemented with 30g/l xylose (n=7, error bars ±SD).

[0025] Figure 8 provides a map of the plasmid PLS0030112.

[0026] Figure 9 provides a map of the plasmid PLS0044980.

DESCRIPTION OF THE INVENTION

[0027] The present invention provides methods and compositions suitable for use in the isomerization of xylose to xylulose, as well as methods and compositions suitable for use in the conversion of xylose to xylitol and xylulose, including nucleic acid constructs, recombinant fungal host cells, and related materials.

[0028] All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference. Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, fermentation, microbiology, and related fields, which are known to those of skill in the art. Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some preferred methods and materials are described. Indeed, it is intended that the present invention not be limited to the particular methodology, protocols, and reagents described herein, as these may vary, depending upon the context in which they are used. The

headings provided herein are not limitations of the various aspects or embodiments of the present invention.

[0029] In order to facilitate understanding of the present invention, a number of terms are defined below. Numeric ranges are inclusive of the numbers defining the range. Thus, every numerical range disclosed herein is intended to encompass every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein. It is also intended that every maximum (or minimum) numerical limitation disclosed herein includes every lower (or higher) numerical limitation, as if such lower (or higher) numerical limitations were expressly written herein.

[0030] As used herein, the term "comprising" and its cognates are used in their inclusive sense (i.e., equivalent to the term "including" and its corresponding cognates).

[0031] As used herein and in the appended claims, the singular "a", "an" and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "host cell" includes a plurality of such host cells.

[0032] Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. The headings provided herein are not limitations of the various aspects or embodiments of the invention that can be had by reference to the specification as a whole. Accordingly, the terms defined below are more fully defined by reference to the specification as a whole.

[0033] As used herein, the terms "isolated" and "purified" are used to refer to a molecule (e.g., an isolated nucleic acid, polypeptide, etc.) or other component that is removed from at least one other component with which it is naturally associated.

[0034] As used herein, the term "reference enzyme" refers to an enzyme to which another enzyme (i.e., the "test enzyme") is compared in order to determine the presence of an improved property in the test enzyme being evaluated, including but not limited to improved activity, thermoactivity, thermostability, and/or stability. In some embodiments, a reference enzyme is a wild-type enzyme (e.g., wild-type xylose isomerase, xylitol dehydrogenase, or xylulokinase). In some embodiments, a reference enzyme is another variant enzyme (e.g., another variant xylose isomerase, xylitol dehydrogenase enzyme, or xylulokinase of the present invention).

[0035] As used herein, the term "recombinant" refers to a polynucleotide or polypeptide that does not naturally occur in a host cell. A recombinant molecule may contain two or more naturally-occurring sequences that are linked together in a way that does not naturally occur. A "recombinant cell" comprises a recombinant polynucleotide or polypeptide.

[0036] As used herein, the term "overexpress" is intended to encompass increasing the production (i.e., expression) of a protein to a level greater than the cell normally produces. It is intended that the term encompass overexpression of endogenous, as well as heterologous proteins.

[0037] As used herein "parent" refers to a starting cell, gene or protein. In some embodiments, "parental strains" are used as the starting point to develop additional strains (e.g., derivatives). In some additional embodiments, "parental molecules" (e.g., "parental enzymes") are used as starting points for evolution/modification to produce variant molecules (e.g., "variant enzymes"). For clarity, reference to a cell of a particular strain refers to a parental cell of the strain as well as progeny and genetically modified derivatives of the same. Genetically modified derivatives of a parental cell include progeny cells that contain a modified genome or episomal plasmids that confer for example, antibiotic resistance, improved fermentation capability, the ability to utilize xylose as a carbon source, etc.

[0038] As used herein, in reference to a specific sequence, the term "modification" encompasses any alteration in a parent amino acid sequence, including but not limited to at least one substitution, deletion, and/or insertion, as well as any change to any component of the sequence. The term also encompasses any alteration in a parent nucleotide sequence, including but not limited to at least one substitution, deletion, insertion, and/or point mutation, etc., (e.g., any change to any component of the sequence). Thus, the term "modification" encompasses the term "mutation," in which a parent nucleotide and/or peptide sequence is altered through any means of mutagenesis.

[0039] A nucleic acid construct, nucleic acid (e.g., a polynucleotide), polypeptide, or host cell is referred to herein as "recombinant" when it is non-naturally occurring, artificial and/or engineered.

[0040] The terms "xylose isomerase" and "xylose isomerase polypeptide" are used interchangeably herein to refer to an enzyme that is capable of catalyzing the isomerization of D-xylose directly to D-xylulose. The ability to catalyze the isomerization of D-xylose directly to D-xylulose is referred to herein as "xylose isomerase activity".

[0041] The terms "xylitol dehydrogenase" and "xylitol dehydrogenase polypeptide" are used interchangeably herein to refer to an enzyme that is capable of catalyzing xylitol to xylulose. The ability to catalyze xylitol to xylulose is referred to herein as "xylitol dehydrogenase activity". Also, as used herein, the term "xylitol dehydrogenase polynucleotide" refers to a polynucleotide that encodes a xylitol dehydrogenase polypeptide.

[0042] The term "xylulokinase" refers to an enzyme that phosphorylates D-xylulose to produce D-xylulose 5-phosphate, which is then further metabolized through the pentose phosphate pathway.

[0043] For example, the term "xylose isomerase polynucleotide" refers to a polynucleotide that encodes a xylose isomerase polypeptide. The term "xylitol dehydrogenase polynucleotide" refers to a polynucleotide that encodes a xylitol dehydrogenase polypeptide. The term "xylulokinase polynucleotide" refers to a polynucleotide that encodes a xylulokinase polypeptide.

[0044] The terms "xylose reductase" and "xylose reductase polypeptide" are used interchangeably herein to refer to an enzyme that is capable of catalyzing xylose to xylitol. The ability to catalyze xylose to xylitol is referred to herein as "xylose reductase activity". The term "xylose reductase polynucleotide" refers to a polynucleotide that encodes a xylose reductase polypeptide.

[0045] The terms "protein" and "polypeptide" are used interchangeably herein to refer to a polymer of amino acid residues. As used herein, the terms "enzyme variant" and "variant enzyme" are used in reference to enzymes that are similar to a reference enzyme, particularly in their function, but have mutations in their amino acid sequence that make them different in sequence from the wild-type or another reference enzyme. Enzyme variants (e.g., "xylose reductase variants," "xylitol dehydrogenase variants," and/or "xylulokinase variants") can be made using any of a wide variety of different mutagenesis techniques well known to those skilled in the art. In addition, mutagenesis kits are also available from many commercial molecular biology suppliers. Methods are available to make specific substitutions at defined amino acids (site-directed), specific or random mutations in a localized region of the gene (regio-specific) or random mutagenesis over the entire gene (e.g., saturation mutagenesis). Numerous suitable methods are known to those in the art to generate enzyme variants, including but not limited to site-directed mutagenesis of single-stranded DNA or double-stranded DNA using PCR, cassette mutagenesis, gene synthesis, error-prone PCR, shuffling, and chemical saturation mutagenesis, or any other suitable method known in the art. After the variants are produced, they can be screened for the desired property (e.g., high or increased; or low or reduced activity, increased thermal and/or alkaline stability, etc.).

[0046] As used herein, "combinatorial variant" refers to any variant that has a combination of two or more mutations (e.g., substitutions). In some embodiments, the combination of mutations results in changes in enzyme activity (e.g., improved thermostability, thermoactivity, and/or specific activity, etc.).

[0047] As used herein, the term "xylose isomerase variant" refers to a xylose isomerase that has been modified from an original starting xylose isomerase. In some embodiments, the term is used in reference to a xylose isomerase polypeptide or polynucleotide encoding a xylose isomerase polypeptide comprising one or more modifications relative to wild-type xylose isomerase or the wild-type polynucleotide encoding xylose isomerase (such as substitutions, insertions, deletions, and/or truncations of one or more amino acid residues or of one or more specific nucleotides or codons in the polypeptide or polynucleotide, respectively), and biologically active fragments thereof. In some embodiments, the xylose isomerase variants are xylose isomerase chimeras.

[0048] The terms "enzyme chimera," "chimeric variant," and "chimeric enzyme" refer to enzymes s that comprise sequences from at least two different parent molecules. In some embodiments, the chimeras are hybrid proteins encoded by nucleotide sequences that have been spliced together from at least two genes. It is not intended that the present invention be limited to any specific number of starting (i.e., "parental" sequences). In some embodiments, the term "chimeric" refers to a nucleic acid, nucleotide sequence and/or encoded product thereof, that contains sequences from two or more different sources. It is contemplated that any suitable source will find use in the present invention, including but not limited to nucleic acid, nucleotide sequence, ribosomal nucleic acid, RNA, DNA, regulatory nucleotide sequences (e.g., promoter, URL, enhancer, repressor, etc.), coding nucleic acid,

gene, nucleic acid linker, nucleic acid tag, amino acid sequence, peptide, polypeptide, protein, chromosome, and/or organism. In some embodiments, "chimeric" molecules include sequences of contiguous nucleotides or amino acids from any suitable source, including but not limited to viruses, prokaryotes, and/or eukaryotes, etc. In some embodiments, chimeras are generated by placing fragments of related and/or unrelated nucleic acids, nucleotide sequences, and/or DNA segments in juxtaposition. In some embodiments, the nucleic acids, nucleotide sequences and/or DNA segments are native (e.g., wild-type) sequences, while in other embodiments, they are mutant and/or engineered (e.g., recombinant) sequences. It is not intended that the present invention be limited to any particular starting component. In some embodiments, the chimera comprises sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 sequences) from one organism and sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 sequences) from at least one other organism (e.g., as contiguous nucleotides or contiguous amino acids). In some embodiments, the organisms are microorganisms, including but not limited to bacteria, yeast, filamentous fungi, etc. In some embodiments, the sequences are obtained from at least two organisms of the same genus and/or species, but of different strains. In some other embodiments, the sequences are obtained from at least two organisms of the same species, while in some other embodiments, the sequence are obtained from at least two organisms of the same genus (i.e., different species). In some embodiments, the chimeras comprise a portion of an enzyme from one bacterial species and at least one additional portion of an enzyme from at least one additional bacterial species. In some embodiments, the chimeras comprise a portion of an enzyme from one fungal species and at least one additional portion of an enzyme from at least one additional fungal species. In some embodiments, the chimeras are comprised of sequences obtained from various types of organisms, for example combinations of bacterial and fungal species, as well as combinations of bacterial, fungal, viral, and/or plant species. Some embodiments of the present invention comprise one portion of an enzyme from a plant, another portion of an enzyme from a bacterium, and another portion of an enzyme from a fungus. Indeed, it is intended that any combination of parental organisms will find use in the present invention. In some embodiments, the chimeras are produced by recombination of two or more nucleotide sequences. Any suitable method for recombination finds use in producing the chimeras. In some embodiments, fragments used to generate chimeras are juxtaposed as units (e.g., nucleotide sequences from the various sources are combined end-to-end and are not interspersed). In some embodiments in which the chimeras include one stretch of contiguous nucleotides per each source organism, nucleotide sequence combinations can be noted as DNA source 1 (1DNA), DNA source 2 (2DNA), etc. (e.g., 1DNA/2DNA etc.), including combinations thereof. In some other embodiments, fragments used to generate the chimeras are interspersed (e.g., 1DNA/2DNA/4DNA/3DNA, etc.). In some embodiments, the chimeric nucleotide sequence encodes activity higher than any of the source nucleotide sequences. In some alternative embodiments, the chimeric nucleotide sequences have similar or same activity as the source nucleotide sequences, but the amount of the activity or kinetics of the activity (e.g., increased or decreased activity), specific activity, and/or other aspects of the

activity are altered. In some additional embodiments, the chimeric nucleotide sequences encode different activities and in some further embodiments, the chimeric nucleotide sequences encode chimeric activities (e.g., a combination of two or more activities).

[0049] In some embodiments, xylose isomerase polynucleotides employed in the practice of the present invention comprise a polynucleotide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical, at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical, at least about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23, and/or a fragment of any of these sequences.

[0050] In some embodiments, xylitol dehydrogenase polynucleotides employed in the practice of the present invention comprise a polynucleotide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70% identical, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical, at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical, at last about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:3, and/or a fragment of any of these sequences.

[0051] In some embodiments, xylulokinase polynucleotides employed in the practice of the present invention comprise a polynucleotide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70% identical, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical, at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical,

at last about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:5, and/or a fragment of any of these sequences.

[0052] In some embodiments, xylose isomerase polypeptides employed in the practice of the present invention comprise a polypeptide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical, at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical, at least about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24, and/or a fragment of any of these sequences.

[0053] In some embodiments, xylitol dehydrogenase polypeptides employed in the practice of the present invention comprise a polypeptide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70% identical, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical, at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical, at last about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:4, and/or a fragment of any of these sequences.

[0054] In some embodiments, xylulokinase polypeptides employed in the practice of the present invention comprise a polypeptide sequence that is at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70% identical, at least about 71% identical, at least about 72% identical, at least about 73% identical, at least about 74% identical, at least about 75% identical, at least about 76% identical, at least about 77% identical, at least about 78% identical, at least about 79% identical,

at least about 80% identical, at least about 81% identical, at least about 82% identical, at least about 83% identical, at least about 84% identical, at least about 85% identical, at least about 86% identical, at last about 87% identical, at least about 88% identical, at least about 89% identical, at least about 90% identical, at least about 91% identical, at least about 92% identical, at least about 93% identical, at least about 94% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, or at least about 99% identical to SEQ ID NO:6, and/or a fragment of any of these sequences.

[0055] The terms "percent identity," "% identity", "percent identical," and "% identical," are used interchangeably herein to refer to the percent amino acid or polynucleotide sequence identity that is obtained by ClustalW analysis (version W 1.8 available from European Bioinformatics Institute, Cambridge, UK), counting the number of identical matches in the alignment and dividing such number of identical matches by the length of the reference sequence, and using the following ClustalW parameters to achieve slow/accurate pairwise optimal alignments – DNA/Protein Gap Open Penalty:15/10; DNA/Protein Gap Extension Penalty:6.66/0.1; Protein weight matrix: Gonnet series; DNA weight matrix: Identity; Toggle Slow/Fast pairwise alignments = SLOW or FULL Alignment; DNA/Protein Number of K-tuple matches:2/1; DNA/Protein number of best diagonals: 4/5; DNA/Protein Window size:4/5.

[0056] Two sequences are "aligned" when they are aligned for similarity scoring using a defined amino acid substitution matrix (*e.g.*, BLOSUM62), gap existence penalty and gap extension penalty so as to arrive at the highest score possible for that pair of sequences. Amino acid substitution matrices and their use in quantifying the similarity between two sequences are well known in the art (*See, e.g.*, Dayhoff *et al.*, *in* Dayhoff [ed.], Atlas of Protein Sequence and Structure," Vol. 5, Suppl. 3, Natl. Biomed. Res. Round., Washington D.C. [1978]; pp. 345-352; and Henikoff *et al.*, Proc. Natl. Acad. Sci. USA, 89:10915-10919 [1992], both of which are incorporated herein by reference). The BLOSUM62 matrix is often used as a default scoring substitution matrix in sequence alignment protocols such as Gapped BLAST 2.0. The gap existence penalty is imposed for the introduction of a single amino acid gap in one of the aligned sequences, and the gap extension penalty is imposed for each additional empty amino acid position inserted into an already opened gap. The alignment is defined by the amino acid position of each sequence at which the alignment begins and ends, and optionally by the insertion of a gap or multiple gaps in one or both sequences so as to arrive at the highest possible score. While optimal alignment and scoring can be accomplished manually, the process is facilitated by the use of a computer-implemented alignment algorithm (*e.g.*, gapped BLAST 2.0; *See*, Altschul *et al.*, Nucleic Acids Res., 25:3389-3402 [1997], which is incorporated herein by reference), and made available to the public at the National Center for Biotechnology Information Website). Optimal alignments, including multiple alignments can be prepared using readily available programs such as PSI-BLAST (*See e.g.*, Altschul *et al.*, *supra*).

[0057] The present invention also provides a recombinant nucleic acid construct comprising a xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotide sequences that hybridize under stringent hybridization conditions to the complement of a polynucleotide which encodes a polypeptide having the amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24. An exemplary polynucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or, is selected from SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23.

[0058] In some embodiments, the polynucleotide that hybridizes to the complement of a polynucleotide which encodes a polypeptide having the amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24, does so under high or very high stringency conditions to the complement of a reference sequence encoding a polypeptide having the sequence of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24 (*e.g.*, over substantially the entire length of the reference sequence).

[0059] Nucleic acids "hybridize" when they associate, typically in solution. There are numerous texts and other reference materials that provide details regarding hybridization methods for nucleic acids (*See e.g.*, Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes," Part1, Chapter 2, Elsevier, New York, [1993], incorporated herein by reference). For polynucleotides of at least 100 nucleotides in length, low to very high stringency conditions are defined as follows: prehybridization and hybridization at 42°C in 5xSSPE, 0.3% SDS, 200 µg/ml sheared and denatured salmon sperm DNA, and either 25% formamide for low stringencies, 35% formamide for medium and medium-high stringencies, or 50% formamide for high and very high stringencies, following standard Southern blotting procedures. For polynucleotides of at least 200 nucleotides in length, the carrier material is finally washed three times each for 15 minutes using 2xSSC, 0.2% SDS at least at 50°C (low stringency), at least at 55°C (medium stringency), at least at 60°C (medium-high stringency), at least at 65°C (high stringency), and at least at 70°C (very high stringency).

[0060] The terms "corresponding to", "with reference to," and "relative to" when used in the context of the numbering of a given amino acid or polynucleotide sequence refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence.

[0061] The terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given polypeptide or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence.

[0062] An amino acid or nucleotide "position" is denoted by a number that sequentially identifies each amino acid or nucleotide in the reference sequence based on its position relative to the N-terminus or 5'-terminus. Owing to deletions, insertions, truncations, fusions, and the like that must be

taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence determined by simply counting from the N-terminus or 5'terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where there is a deletion in an aligned test sequence, there will be no amino acid that corresponds to a position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference sequence, that insertion will not correspond to any amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence. As used herein, in referring to variants (*e.g.*, variants with substitutions, insertions, and/or deletions), a hyphen indicates a deletion in a sequence and an asterisk indicates a mutation in a stop codon.

[0063] As used herein, the term "conservative substitution" refers to the substitution of a residue for another residue that does not generally alter the specific activity of the encoded polypeptide. In some embodiments, a "conservative substitution," as used with respect to amino acids, refers to the substitution of an amino acid with a chemically similar amino acid. An exemplary conservative substitution is a substitution that is within the same group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine, proline, cysteine and methionine). Amino acid substitutions which often preserve the structural and/or functional properties of the polypeptide in which the substitution is made are well known in the art Amino acid substitutions that do not generally alter the specific activity are known in the art (*See e.g.*, Neurath and Hill, The Proteins, Academic Press, New York [1979], which is incorporated herein by reference). Some of the most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, His/Asn, His/Gln, Lys/Asn, Lys/Gln, Lys/Gln, Tyr/Phe, Tyr/His, Tyr/Tarp, Ala/Pro, Lys/Arg, Gln/Arg, Asp/Asn, Leu/Ile, Leu/Val, Leu/Met, Ile/Met, Ala/Glu, Glu/Gln, Phe/Leu, Phe/Met, Val/Met, and Asp/Gly, as well as these in reverse.

[0064] The following nomenclature finds use in describing substitutions in a reference sequence relative to a reference sequence or a variant polypeptide or nucleic acid sequence: "R-#-V," where "#" refers to the position in the reference sequence, "R" refers to the amino acid (or base) at that position in the reference sequence, and "V" refers to the amino acid (or base) at that position in the variant sequence. In some embodiments, an amino acid (or base) may be called "X," by which is meant any amino acid (or base). As a non-limiting example, for a variant polypeptide described with reference to SEQ ID NO:2, "E10G" indicates that in the variant polypeptide, the glutamic acid at position 10 of the reference sequence is replaced by glycine, with amino acid position being determined by optimal alignment of the variant sequence with SEQ ID NO:2. Similarly, "E10G/D" describes two variants: a variant in which the glutamic acid at position 10 of the reference sequence is replaced by glycine; and

a variant in which the glutamic acid at position10 of the reference sequence is replaced by aspartic acid.

[0065] As used herein, the terms "amino acid substitution set" and "substitution set" when used in the context of amino acid sequences (*e.g.*, polypeptides) refer to a group of (*i.e.*, multiple) amino acid substitutions.

[0066] As used herein, the terms "amino acid mutation set" and "mutation set" when used in the context of amino acid sequences (*e.g.*, polypeptides) refer to a group of (*i.e.*, multiple) amino acid substitutions, insertions, and/or deletions.

[0067] As used herein, the terms "nucleic acid substitution set" and "substitution set" when used in the context of nucleotide sequences (*e.g.*, polynucleotides) refer to a group of (*i.e.*, multiple) nucleic acid substitutions.

[0068] As used herein, the terms "nucleic acid mutation set" and "mutation set" when used in the context of nucleotide sequences (*e.g.*, polynucleotides) refer to a group of (*i.e.*, multiple) nucleic acid substitutions, insertions, and/or deletions.

[0069] As used herein, the terms "host cell" and "host strain" refer to suitable hosts for expression vectors comprising DNA provided herein. In some embodiments, the host cells are prokaryotic or eukaryotic cells that have been transformed or transfected with vectors constructed using recombinant DNA techniques as known in the art. Transformed hosts are capable of either replicating vectors encoding at least one protein of interest and/or expressing the desired protein of interest. In addition, reference to a cell of a particular strain refers to a parental cell of the strain as well as progeny and genetically modified derivatives. Genetically modified derivatives of a parental cell include progeny cells that contain a modified genome or episomal plasmids that confer for example, antibiotic resistance, improved fermentation, etc. In some embodiments, host cells are genetically modified to have characteristics that improve protein secretion, protein stability or other properties desirable for expression and/or secretion of a protein. Genetic modification can be achieved by any suitable genetic engineering techniques and/or classical microbiological techniques (*e.g.*, chemical or UV mutagenesis and subsequent selection). Using recombinant technology, nucleic acid molecules can be introduced, deleted, inhibited or modified, in a manner that results in increased yields of enzyme(s) of interest within the organism or in the culture. In some genetic engineering approaches, homologous recombination is used to induce targeted gene modifications by specifically targeting a gene *in vivo* to suppress expression of the encoded protein. In an alternative approach, siRNA, antisense, and/or ribozyme technology finds use in inhibiting gene expression.

[0070] As used herein, the term "transformed" or "transformation" used in reference to a cell means that the cell has a non-native nucleic acid sequence integrated into its genome or has an episomal plasmid that is maintained through multiple generations.

[0071] As used herein, the term "by-product" refers to an organic molecule that is an undesired product of a particular fermentation process.

[0072] As used herein, the term "xylose pathway" refers to the steps of conversion of xylose to xylulose phosphate which is then metabolized through the pentose phosphate pathway. In some embodiments, this involves the reduction of xylose to xylitol, oxidation of xylitol to xylulose and subsequent conversion of xylulose to xylulose phosphate. In some other embodiments the xylose is directly converted to xylulose which is then phosphorylated to xylulose phosphate.

[0073] As used herein the term "xylose pathway enzymes" refers to the enzymes that catalyze the conversion of xylose to xylulose phosphate which is then metabolized through the pentose phosphate pathway. In some embodiments, these enzymes comprise xylose reductase, xylitol dehydrogenase and/or xylulose kinase. In some other embodiments, the enzymes comprise xylose isomerase and xylulose kinase. In some additional embodiments, the enzymes comprise xylose isomerase, xylitol dehydrogenase, and xylulokinase.

DETAILED DESCRIPTION OF THE INVENTION

[0074] The present invention provides methods and compositions suitable for use in the isomerization of xylose to xylulose, as well as methods and compositions suitable for use in the conversion of xylose to xylitol and xylulose, including nucleic acid constructs, recombinant fungal host cells, and related materials.

[0075] The initial metabolic pathways for xylose utilization in fungi and bacteria differ. In most fungi, including xylose-fermenting yeasts (*e.g.*, *Pichia stipitis*, *Pachysolen tannophilus*, and *Candida shehatae*), D-xylose is converted to D-xylulose by two oxidoreductases involving cofactors NAD(P)H and NAD(P)+ (*See,* Matsushika *et al.*, Appl. Microbiol. Biotechnol., 84:37-53 [2009]). In these organisms, D-xylose is initially reduced to xylitol by NAD(P)H-dependent xylose reductase (XR) (EC 1.1.1.21). Xylitol is subsequently oxidized to D-xylulose by NAD+-dependent xylitol dehydrogenase (XDH) (EC 1.1.1.9). Xylulokinase (XK) (EC 2.7.1.17) subsequently phosphorylates D-xylulose to produce D-xylulose 5-phosphate (X5P), which is then further metabolized through the pentose phosphate pathway (PPP).

[0076] However, most strains of *S. cerevisiae* cannot utilize xylose even though the genes encoding XR, XDH, and XK are present in its genome, as the expression levels of these enzymes are too low to allow xylose utilization (*See,* Matsushika *et al.*, *supra*). Some strains have been shown to natively utilize xylose but at very low rates and fermentation to ethanol has not been detected (*See,* Wenger *et al.*, PLoS Genet., 6(5):e1000942 [2010]). Even when the endogenous genes are overexpressed in *S. cerevisiae,* only slow growth on xylose has been observed (*See,* Matsushika *et al.*, *supra*).

[0077] In contrast, most bacteria (*e.g.*, *Escherichia coli* and *Streptomyces* species) can isomerize D-xylose directly to D-xylulose by using a xylose isomerase (XI) (EC 5.3.1.5) (*See,* Matsushika *et al.*, *supra*). In bacteria, as in fungi, the D-xylulose is phosphorylated to D-xylulose 5-phosphate by XK, which is then further metabolized through the pentose phosphate pathway.

[0078] Efforts to express a functional heterologous xylose isomerase gene (*xylA*) in *S. cerevisiae* and grow the yeast on xylose has met with very limited success (*See e.g.*, Matsushika *et al. supra*). It has been reported that xylose isomerase genes from the fungi *Piromyces* (Kuyper *et al.* FEMS Yeast Res., 4:69-78 [2003]) and *Orpinomyces* (Madhaven *et al.*, Appl. Microbiol. Biotechnol., 82:1067-1078 [2009a]) have been functionally expressed in *S. cerevisiae*, but that growth on xylose was very slow. In addition, the functional expression of the *Thermus thermophilus* xylose isomerase (Accession No. 1BXB) in *S. cerevisiae* has been reported (*See*, Walfridsson *et al.*, Appl. Environ. Microbiol., 62:4648-4651 [1996]). The success in producing an active xylose isomerase by expressing the *T. thermophilus xylA* gene in *S. cerevisiae* may have been due to the relatedness between the two organisms, as *T. thermophilus* diverged from the domain of eubacteria and may, in many respects, be more closely related to *S. cerevisiae* than are the eubacteria (*Id.*, at 4651).

[0079] Heterologous expression of xylose isomerase genes from *Actinoplanes missouriensis* and *Clostridium thermosulfurogenes* in *S. cerevisiae* generated inactive proteins, even though their messenger RNA could be detected (*See*, Amore *et al.*, Appl. Microbiol. Biotechnol., 30:351-357 [1989]); and Moes *et al.*, Biotech. Lett., 18:269-274 [1996]; and Matsushika *et al.*, *supra*). Other studies report the heterologous expression of the *xylA* from *E. coli* (*See e.g.*, Sarthy *et al.*, Appl. Environ. Microbiol., 53:1996-2000 [1987]), *Bacillus subtilis* (Amore *et al.*, Appl. Microbiol. Biotechnol., 30:351-357 [1989]), and *Streptomyces rubiginosus* (Gárdonyi *et al.*, Enzyme Microb. Technol., 32:252-259 [2003]) in *S. cerevisiae* resulted in mainly insoluble proteins which were catalytically inactive (*See*, Matsushika *et al.*, *supra*). In addition, some reports indicate that attempts to produce xylose isomerase from recombinant *S. cerevisiae* transformed with the *xylA* genes from *Bacillus subtilis* and *Lactobacillus pentosus* resulted in inactive protein (*See*, Walfridsson *et al.*, *supra*).

[0080] In further studies, the results of screening for xylose isomerase activity in *S. cerevisiae* transformed with the xylose isomerase genes from various organisms have been reported (*See e.g.*, Brat *et al.*, Appl. Environ. Microbiol. Doi:10.1128/AEM.02522-9 [13 February 2009]). The xylose isomerases have been reported to share from 17% to 60% sequence identity to the xylose isomerase from *Piromyces*. While transformants expressing the xylose isomerase from *Clostridium phytofermentans* (DSM 18823) could grow on xylose medium, *S. cerevisiae* transformed with the xylose isomerase gene from the following organisms could not: *Bacillus licheniformis* (DSM 13), *Burkholderia xenovaorans* (DSM 17367), *Lactobacillus pentosus* (DSM 20314), *Leifsonia xyli* subsp. *cynodontis* (DSM 46306), *Pseudomonas savastanoi* pvar. *Phaseolicola* (DSM 50282), *Robiginitalea biformata* (DSM 15991), *Saccharophagus degradans* (DSM 17024), *Staphylococcus xylosus* (DSM 20266), *Streptomyces diastaticus* subsp. *diastaticus* (DSM 40496), *Xanthomonas campestris* var. *campestris* (DSM 3586), *Salmonella typhimurium* (71-098L), *Agrobacterium tumefaciens*, and *Arabidopsis thaliana* (*See*, Brat *et al.*, *supra*).

[0081] The present invention provides sequences that are capable of conferring the property of xylose-utilization in a non-mammalian, eukaryotic host cell, such as, for example, a fungal host cell. These sequences and variants thereof, encode xylose isomerases, which catalyze the isomerization of D-xylose directly to D-xylulose, as depicted in Figure 1. Xylose isomerase is distinguished from xylose reductase (XR), which catalyzes the conversion of xylose to xylitol. Xylose isomerase is also distinguished from xylitol dehydrogenase (XD), which catalyzes the conversion of xylitol to D-xylulose (*See*, Figure 1).

[0082] Xylose utilization by these host cells results in useful products that are produced metabolically by the host cell. In these host cells, D-xylulose may be phosphorylated by a native or recombinant xylulokinase to xylulose-5-P, as depicted in Figure 1. The xylulose-5-P may be further metabolized by enzymes in the pentose phosphate pathway to products such as glucose-6-P, fructose-6-P, glyceraldehydes-3-P, and the like. The pentose phosphate pathway and relevant enzymes and products are depicted in Figure 2A. As used herein, the terms "enzyme from the pentose phosphate pathway" and "pentose phosphate pathway enzyme" are used interchangeably to refer to an enzyme from the group of enzymes involved in the pentose phosphate pathway, (*i.e.*, 6. ribulose-5-phosphate ketoisomerase (RKI1); 7. transketolase (TKL1); 8. transaldolase (TAL1); 9. ribose-5-phosphate ketolisomerase (RKI1); 10. 6-phosphogluconate dehydrogenase (GND1); 11. 6-phosphogluconalactonase (SOL3); and/or 12. glucose-6-phosphate-1-dehydrogenase (ZWF); the reference numbers correspond to those in Figure 2A).

[0083] Products of the pentose phosphate pathway may be further metabolized through the process of glycolysis. The metabolic process of glycolysis is depicted in Figure 2B. As used herein, the term "glycolytic enzyme" refers to an enzyme from the group of enzymes involved in glycolysis (*i.e.*: 13. hexokinase; 14. phosphoglucose isomerase; 15. phosphofructokinase; 16. aldolase; 17. triose phosphate isomerase; 18. glyceraldehyde phosphate dehydrogenase; 19. phosphoglycerate kinase; 20. phosphoglyceromutase; 21. enoase; and/or 22. pyruvate kinase; the reference numbers correspond to those in Figure 2B).

[0084] Pyruvate from the glycolytic pathway (*i.e.*, glycolysis) may be further metabolized to ethanol as shown in Figure 2C by ethanologenic enzymes. As used herein, the term "ethanologenic enzyme" refers to an enzyme involved in the conversion of pyruvate to ethanol, (*e.g.*, a pyruvate decarboxylase, an aldehyde dehydrogenase, and/or an alcohol dehydrogenase). The term "ethanologenic pathway" refers to the pathway depicted in Figure 2C.

[0085] The polynucleotide sequences described herein are useful for creating recombinant fungal host cells, particularly yeast host cells, that are capable of isomerizing D-xylose directly to D-xylulose, which can lead to the production of desirable fermentation products. Recombinant host cells transformed with xylose reductase and xylitol dehydrogenase genes are hence capable of converting xylose to xylitol and then converting xylitol to xylulose, which can lead to the production of desirable fermentation products (*e.g.*, an alcohol, such as ethanol, butanol, and the like, including,

but not limited to a fatty alcohol [*e.g.*, a C8-C20 fatty alcohol], a fatty acid [*e.g.*, a C8-C20 fatty acid], lactic acid, 3-hydroxpropionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, a β-lactam, and the like). However, previous reports have indicated that cells transformed with wild-type xylose reductase and xylitol dehydrogenase genes from *Pichia stipitis* convert xylose inefficiently and with accumulation of xylitol (Matsushika *et al.*, Appl. Environ Microbiol., 81:243-55 [2008]).

[0086] In contrast, recombinant host cells transformed with xylose isomerase, xylitol dehydrogenase, and xylulokinase genes as described herein, are capable of converting xylose to desirable fermentation products (*e.g.*, an alcohol, such as ethanol, butanol, and the like, including, but not limited to a fatty alcohol [*e.g.*, a C8-C20 fatty alcohol], a fatty acid [*e.g.*, a C8-C20 fatty acid], lactic acid, 3-hydroxpropionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, a β-lactam, and the like). In some embodiments, the recombinant host cells are further transformed with genes encoding xylose reductase.

Recombinant Nucleic Acid Constructs

[0087] In some embodiments, the present invention provides recombinant nucleic acid constructs comprising a polynucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:4; and/or a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about

96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:6. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation.

[0088] The present invention provides recombinant nucleic acid constructs comprising polynucleotide sequences that encode at least one polypeptide comprising amino acid sequences having 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 at least 100% identity to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation.

[0089] In some embodiments, the present invention provides recombinant nucleic acid constructs comprising a polynucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at

least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation.

[0090] The present invention provides recombinant nucleic acid constructs comprising polynucleotide sequences that encode a polypeptide comprising amino acid sequences having at least 70%, % 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 at least 100% identity to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation.

[0091] The present invention also provides nucleic acid constructs comprising polynucleotides encoding at least one xylose reductase, xylitol dehydrogenase and/or xylulokinase. The present invention further provides nucleic acid constructs comprising polynucleotides encoding at least one xylose reductase, xylitol dehydrogenase and xylulokinase. In some of these embodiments, the nucleic acid constructs comprise SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; SEQ ID NO:3; and/or SEQ ID NO:5. In some additional embodiments, the nucleic acid constructs comprise SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; SEQ ID NO:3; and SEQ ID NO:5.

[0092] In some embodiments, recombinant nucleic acid constructs of the present invention further comprise at least one polynucleotide sequence (i.e., genetic) element that facilitates integration into a fungal host cell genome, by homologous or non-homologous recombination. In some embodiments, the nucleic acid construct of the present invention further comprises an origin of replication that is functional in a fungal cell (*e.g.*, a yeast origin of replication). Typically, the fungal host cell is a yeast or filamentous fungal cell, more typically, a yeast cell. In some embodiments, nucleic acid constructs of the present invention comprise at least one transcriptional regulatory element that is functional in a fungal cell. For example, in some embodiments the recombinant nucleic acid construct comprises at least one promoter sequence and/or transcription terminator sequence that is functional in a fungal cell such that the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide is operatively linked to the promoter sequence and/or transcription terminator sequences. In some additional embodiments the recombinant nucleic acid construct comprises at least one promoter sequence and/or transcription terminator sequence that is functional in a fungal cell such that the xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotide are operatively linked to the promoter sequence and/or transcription terminator sequences.

[0093] Additional xylose isomerase, xylose reductase, xylitol dehydrogenase, and xylulokinase polynucleotides suitable for use in the practice of the present invention include those encoding variants generated by mutagenesis, recombination, and/or any other protein engineering method. In some embodiments, the variants are screened for xylose utilization using any suitable method as known in the art. In some embodiments, the resulting variants comprise one or more substitutions (conservative or non-conservative), deletions, and/or insertions.

[0094] Methods for generating variant libraries of polynucleotides encoding modified polypeptides are well known in the art. For example, mutagenesis and directed evolution methods can be readily applied to polynucleotides encoding xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptides to generate variant libraries that can be expressed, screened, and assayed using any suitable methods known in the art. Mutagenesis and directed evolution methods are well known in the art (*See e.g.*, US Patent Nos. 5,605,793, 5,830,721, 6,132,970, 6,420,175, 6,277,638, 6,365,408, 6,602,986, 7,288,375, 6,287,861, 6,297,053, 6,576,467, 6,444,468, 5,811238, 6,117,679, 6,165,793, 6,180,406, 6,291,242, 6,995,017, 6,395,547, 6,506,602, 6,519,065, 6,506,603, 6,413,774, 6,573,098, 6,323,030, 6,344,356, 6,372,497, 7,868,138, 5,834,252, 5,928,905, 6,489,146, 6,096,548, 6,387,702, 6,391,552, 6,358,742, 6,482,647, 6,335,160, 6,653,072, 6,355,484, 6,03,344, 6,319,713, 6,613,514, 6,455,253, 6,579,678, 6,586,182, 6,406,855, 6,946,296, 7,534,564, 7,776,598, 5,837,458, 6,391,640, 6,309,883, 7,105,297, 7,795,030, 6,326,204, 6,251,674, 6,716,631, 6,528,311, 6,287,862, 6,335,198, 6,352,859, 6,379,964, 7,148,054, 7,629,170, 7,620,500, 6,365,377, 6,358,740, 6,406,910, 6,413,745, 6,436,675, 6,961,664, 7,430,477, 7,873,499, 7,702,464, 7,783,428, 7,747,391, 7,747,393, 7,751,986, 6,376,246, 6,426,224, 6,423,542, 6,479,652, 6,319,714, 6,521,453, 6,368,861, 7,421,347, 7,058,515, 7,024,312, 7,620,502, 7,853,410, 7,957,912, 7,904,249, and all related non-US counterparts; Ling *et*

al., Anal. Biochem., 254(2):157-78 [1997]; Dale *et al.*, Meth. Mol. Biol., 57:369-74 [1996]; Smith, Ann. Rev. Genet., 19:423-462 [1985]; Botstein *et al.*, Science, 229:1193-1201 [1985]; Carter, Biochem. J., 237:1-7 [1986]; Kramer *et al.*, Cell, 38:879-887 [1984]; Wells *et al.*, Gene, 34:315-323 [1985]; Minshull *et al.*, Curr. Op. Chem. Biol., 3:284-290 [1999]; Christians *et al.*, Nat. Biotechnol., 17:259-264 [1999]; Crameri *et al.*, Nature, 391:288-291 [1998]; Crameri, *et al.*, Nat. Biotechnol., 15:436-438 [1997]; Zhang *et al.*, Proc. Nat. Acad. Sci. U.S.A., 94:4504-4509 [1997]; Crameri *et al.*, Nat. Biotechnol., 14:315-319 [1996]; Stemmer, Nature, 370:389-391 [1994]; Stemmer, Proc. Nat. Acad. Sci. USA, 91:10747-10751 [1994]; WO 95/22625; WO 97/0078; WO 97/35966; WO 98/27230; WO 00/42651; WO 01/75767; and WO 2009/152336, all of which are incorporated herein by reference).

[0095] Also suitable for use in the practice of the present invention are polynucleotides encoding a truncated xylose isomerase, xylitol dehydrogenase, and/or xylulokinase or sequence variant(s) thereof. These truncation variants may be truncated at the carboxy (C)-terminus and/or the amino (N)-terminus. Typically, the truncation is from about 1 to about 50 amino acid residues. However, it not intended that the present invention be limited to any specific number of truncated amino acid residues.

[0096] Those having ordinary skill in the art will understand that due to the degeneracy of the genetic code, a multitude of nucleotide sequences that encode the xylose isomerase, xylitol dehydrogenase, and xylulokinase polypeptides described herein exist. Table 1 provides the standard triplet genetic code for each amino acid. For example, the codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acids referred to herein, where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. It is understood that U in an RNA sequence corresponds to T in a DNA sequence. The invention contemplates and provides each and every possible variation of nucleic acid sequence encoding a polypeptide of the invention that could be made by selecting combinations based on possible codon choices.

Table 1. Genetic Code

Amino Acid	3 Letter Code	Single Letter Code	Codon(s)
Alanine	Ala	A	GCA, GCC, GCG, GCU
Cysteine	Cys	C	UGC, UGU
Aspartic acid	Asp	D	GAC, GAU
Glutamic acid	Glu	E	GAA, GAG
Phenylalanine	Phe	F	UUC, UUU
Glycine	Gly	G	GGA, GGC, GGG, GGU
Histidine	His	H	CAC, CAU
Isoleucine	Ile	I	AUA, AUC, AUU
Lysine	Lys	K	AAA, AAG
Leucine	Leu	L	UUA, UUG, CUA, CUC, CUG, CUU

Methionine	Met	M	AUG
Asparagine	Asn	N	AAC, AAU
Proline	Pro	P	CCA, CCC, CCG, CCU
Glutamine	Gln	Q	CAA, CAG
Arginine	Arg	R	AGA, AGG, CGA, CGC, CGG, CGU
Serine	Ser	S	AGC, AGU, UCA, UCC, UCG, UCU
Threonine	Thr	T	ACA, ACC, ACG, ACU
Valine	Val	V	GUA, GUC, GUG, GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC, UAU

[0097] In some embodiments, DNA sequences are designed for high codon usage bias (*i.e.*, codons that are used at higher frequency in the protein coding regions than other codons that code for the same amino acid). The preferred codons may be determined in relation to codon usage in a single gene, a set of genes of common function or origin, highly expressed genes, the codon frequency in the aggregate protein coding regions of the whole organism, codon frequency in the aggregate protein coding regions of related organisms, or combinations thereof. Codons whose frequency increases with the level of gene expression are typically optimal codons for expression. In particular, a DNA sequence can be optimized for expression in a particular host organism. References providing preference information for a wide range of organisms are readily available and known in the art (*See e.g.*, Henaut and Danchin *in* Neidhardt *et al.* [eds.], *Escherichia coli* and *Salmonella*, ASM Press, Washington D.C., [1987], p. 2047-2066, which is incorporated herein by reference).

[0098] A variety of methods are known for determining the codon frequency (*e.g.*, codon usage, relative synonymous codon usage) and codon preference in specific organisms, including multivariate analysis, for example, using cluster analysis or correspondence analysis, and the effective number of codons used in a gene (*See*, GCG CodonPreference, Genetics Computer Group Wisconsin Package;, Peden, Codon W, University of Nottingham; McInerney, Bioinform.,14:372-73 [1998]; Stenico *et al.*, Nucl. Acids Res. 222437-46 [1994]; Wright, Gene 87:23-29 [1990]; Wada *et al.*, Nucl. Acids Res., 20:2111-2118 [1992]; Nakamura *et al.*, Nucl. Acids Res., 28:292 [2000]; and Henaut and Danchin, *supra*; all of which are incorporated herein by reference). The data source for obtaining codon usage may rely on any available nucleotide sequence capable of coding for a protein. These data sets include nucleic acid sequences actually known to express proteins (*e.g.*, complete protein coding sequences-CDS), expressed sequence tags (ESTs), or predicted coding regions of genomic sequences (*See* e.g., Mount, Bioinformatics: Sequence and Genome Analysis, Chapter 8, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, [2001]; Uberbacher, Methods Enzymol., 266:259-281 [1996]; and Tiwari *et al.*, Comput. Appl. Biosci. 13:263-270 [1997]; all of which are incorporated herein by reference). It is not intended that the present invention be limited to any particular method, data source and/or data set.

[0099] In some embodiments, the xylose isomerase, xylitol dehydrogenase and/or xylulokinase polynucleotide contains codons optimized for expression in a fungal cell, particularly a yeast cell. In some embodiments, silent mutations (i.e., DNA mutations that do not affect the amino acid sequence of the protein) are also present. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotides are employed in recombinant nucleic acid constructs that comprise a vector (*e.g.*, a plasmid, a cosmid, a phage, a virus, a yeast artificial chromosome [YAC], and the like), into which xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide sequence(s) has/have been inserted. The xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotides provided herein find use incorporated into any one of a variety of vectors. Suitable vectors include, but are not limited to chromosomal, nonchromosomal and synthetic DNA sequences, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and many others. Indeed, any suitable vector that transduces genetic material into a cell, and, if replication is desired, that is replicable and viable in the relevant host find use in the present invention.

[0100] Nucleic acid constructs of the present invention find use in transforming a host cell to permit the host to express the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptide(s). Methods for recombinant expression of proteins in fungi are well known in the art, and a number of vectors are available or can be constructed using routine methods (*See e.g.*, Zhu *et al.*, Plasmid 6:128-33 [2009], incorporated herein by reference; and the many standard references in this field).

[0101] In some embodiments, recombinant nucleic acid constructs of the present invention further comprise a transcriptional regulatory element that is functional in a fungal cell. In some embodiments, the nucleic acid construct comprises the xylose isomerase, xylitol dehydrogenase and/or xylulokinase polynucleotide(s) operatively linked to a transcriptional regulatory sequence (*e.g.*, a promoter, transcription termination sequence, and the like), that is functional in a fungal cell. Examples of promoters that are functional in a fungal host cell include, but are not limited to promoters from yeast and filamentous fungi. Promoters that are suitable for use in the practice of the present invention include endogenous or heterologous promoters and include both constitutive and inducible promoters that are natural or modified. Particularly useful promoters are those that are insensitive to catabolite (glucose) repression and/or do not require xylose for induction. Such promoters are well known in the art. In some embodiments, a promoter sequence is operably linked to the 5' region of the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase coding sequence using routine methods that are well known in the art.

[0102] Promoters that are suitable for use in the practice of the present invention include, but are not limited to yeast promoters from glycolytic genes (*e.g.*, yeast phosphofructokinase (PFK), triose phosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GPD, TDH3 or GAPDH), pyruvate kinase (PYK), phosphoglycerate kinase (PGK) promoters, and the like; *See e.g.*, WO 93/03159, incorporated herein by reference); promoters of glucose transporters; ribosomal protein

encoding gene promoters; alcohol dehydrogenase promoters (*e.g.*, ADH1, ADH4, and the like), and the enolase promoter (ENO).

[0103] Exemplary promoters useful for directing the transcription of the nucleic acid constructs of the present invention in yeast host cells include, but are not limited to those from the genes for *Saccharomyces cerevisiae* enolase (eno-1), *Saccharomyces cerevisiae* galactokinase (*gal*1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1/ADH2/GAP), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* transcription elongation factor (TEF), *Saccharomyces cerevisiae* fructose 1,6-bisphosphate aldolase (FBA1), and *Saccharomyces cerevisiae* 3-phosphate glycerate kinase (PGK1). Other useful promoters for yeast host cells are well known in the art (*See e.g.*, Romanos *et al.*, Yeast 8:423-488 [1992], incorporated herein by reference).

[0104] Suitable filamentous fungal promoters useful in the practice of the present invention include, but are not limited to promoters obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, and *Fusarium oxysporum* trypsin-like protease (*See e.g.*, WO 96/00787, which is incorporated herein by reference), as well as the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase), promoters such as *cbh1*, *cbh2*, *egl*1, *egl*2, *pepA*, *hfb1*, *hfb2*, *xyn1*, *amy*, and *glaA* (*See*, Nunberg *et al.*, Mol. Cell Biol., 4:2306-2315 [1984]; Boel *et al.*, EMBO J. 3:1581-85 [1984]; and EP 0 137 280A, all of which are incorporated herein by reference), and mutant, truncated, and hybrid promoters thereof. Promoters associated with chitinase production in fungi also find use in some embodiments (*See e.g.*, Blaiseau and Lafay, Gene 120:243-248 [1992] [filamentous fungus *Aphanocladium album*]; and Limon *et al.*, Curr. Genet., 28:478-83 [1995] [*Trichoderma harzianum*]; both of which are incorporated herein by reference).

[0105] Any other suitable promoter sequence that drives expression in a fungal host cell, particularly a yeast host cell finds use in the present invention. Suitable promoter sequences can be identified using well known methods. In one approach, a putative promoter sequence is linked 5' to a sequence encoding a reporter protein, the construct is transfected into the host cell and the level of expression of the reporter is measured. Expression of the reporter can be determined by measuring, for example, mRNA levels of the reporter sequence, an enzymatic activity of the reporter protein, or the amount of reporter protein produced. For example, promoter activity may be determined by using the green fluorescent protein as coding sequence (*See e.g.*, Henriksen *et al.*, Microbiol., 145:729-34 [1999], which is incorporated herein by reference) or a lacZ reporter gene (*See e.g.*, Punt *et al.*, Gene, 197:189-93 [1997], which is incorporated herein by reference). In some embodiments, functional promoters are derived from naturally occurring promoter sequences by directed evolution methods

(*See e.g.*, Wright *et al.*, Hum. Gene Ther., 16:881-892 [2005], which is incorporated herein by reference).

[0106] In some embodiments, heterologous and/or recombinant transcription termination sequences find use in the present invention. There are various exemplary transcription termination sequences (terminators) functional in fungal host cells, include transcription termination sequences from yeast and filamentous fungi well known in the art. In some embodiments, the transcription termination sequence is a yeast sequence. Exemplary yeast transcription termination sequences include, but are not limited to CYC1, ADH1t, ADH2t, etc. In some embodiments, the nucleic acid constructs of the present invention contain a ribosome binding site for translation initiation. In some embodiments, the construct includes appropriate sequences for amplifying expression (*e.g.*, an enhancer). Such elements are well known in the art and any suitable enhancers and/or transcription termination sequences, and/or ribosome binding sites find use in the present invention.

[0107] In some additional embodiments, nucleic acid constructs of the present invention contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Suitable marker genes include, but are not limited to those coding for antimicrobial resistance such as, ampicillin (ampR), kanamycin, chloramphenicol, tetracycline, streptomycin or spectinomycin (*e.g.*, the aada gene); including but not limited to the streptomycin phosphotransferase (*spt*) gene coding for streptomycin resistance, the neomycin phosphotransferase (*nptII*) gene encoding kanamycin or geneticin resistance, the nourseothricin acetyltransferase (nat1) gene coding for nourseothricin resistance, the hygromycin phosphotransferase (*hpt*) gene coding for hygromycin resistance, genes encoding dihydrofolate reductase, phleomycin, or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance in *E. coli*, as well as other marker genes that are well known in the art. Indeed, any suitable marker gene finds use in the present invention.

[0108] In some embodiments, nucleic acid constructs of the present invention typically comprise a fungal origin of replication (e.g., a filamentous fungal or yeast origin of replication). In some embodiments, the recombinant nucleic acid constructs of the present invention comprise a yeast origin of replication. Examples include, but are not limited to constructs containing autonomous replicating sequences, constructs containing 2 micron DNA including the autonomous replicating sequence and *rep* genes, constructs containing centromeres like the CEN6, CEN4, CEN11, CDN3 and autonomous replicating sequences, and other like sequences that are well known in the art. Exemplary nucleic acid constructs include constructs suitable for transforming yeast. These include, but are not limited to episomal constructs based on the yeast 2μ or CEN origin based plasmids like pYES2/CT, pYES3/CT, pESC/His, pESC/Ura, pESC/Trp, pES/Leu, p427TEF, pRS405, pRS406, pRS413, and other yeast-based constructs that are known in the art. Indeed, any suitable origin of replication finds use in the present invention.

[0109] In some embodiments, the nucleic acid constructs of the present invention comprise elements to facilitate integration of the xylose isomerase, xylitol dehydrogenase and/or xylulokinase

polynucleotide(s) into a fungal host chromosome (*i.e.*, the genome), by either homologous or non-homologous recombination and/or either site-directed and/or random mutagenesis. In some embodiments, the nucleic acid constructs comprise elements that facilitate homologous integration. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide is integrated at one or more sites and is present in one or more copies. In some embodiments, the nucleic acid construct comprises the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide(s) and no promoter that is operatively linked to the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide(s). This type of construct typically comprises genetic elements to facilitate integration into the fungal host chromosome at a location that is downstream of a native promoter (*i.e.*, in the host chromosome). In some embodiments, a second nucleic acid comprising a promoter and genetic elements to facilitate integration into the fungal host chromosome in a location upstream of the targeted integration site of the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide finds use. In some embodiments, the nucleic acid construct comprises the xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotides operatively linked to a promoter or promoter and terminator sequences such that all are integrated into the host chromosome (genome). It is contemplated that any suitable element that facilitates integration will find use in the present invention.

[0110] Genetic elements that facilitate integration by homologous recombination include those having sequence homology to targeted integration sites in the fungal host chromosome (genome). Suitable sites that find use as targets for integration include, but are not limited to the TY1 loci, the RDN loci, the ura3 locus, the GPD locus, aldose reductase (GRE3) locus, etc. Those having ordinary skill in the art will appreciate that additional sites for integration can be readily identified using methods known in the art, including but not limited to microarray analysis, metabolic flux analysis, comparative genome hybridization analysis, etc.

[0111] Genetic elements or techniques that facilitate integration by non-homologous recombination include, but are not limited to restriction enzyme-mediated integration (REMI) (*See e.g.*, Manivasakam *et al.*, Mol. Cell Biol., 18(3):1736-1745 [1998], incorporated herein by reference), transposon-mediated integration, and other elements and methods that are well known in the art. Indeed, any suitable method that facilitates homologous and/or non-homologous recombination finds use in the present invention.

[0112] In some embodiments, the nucleic acid constructs of the present invention comprise at least one further recombinant polynucleotide that is capable of conferring a desired phenotype to a fungal host cell, particularly in the context of xylose fermentation. In some embodiments, the recombinant polynucleotide that is capable of conferring an improved phenotype to the fungal host cell is a non-coding polynucleotide (e.g., a regulatory polynucleotide), a coding polynucleotide, or combination thereof.

[0113] Exemplary further desired phenotypes include, but are not limited to increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to increased osmolarity, increased tolerance to organic acids, reduced production of by-products, and other similar properties related to increasing flux through the pentose phosphate and glycolysis pathways to produce a desired metabolic product/intermediate at higher levels as compared to the corresponding wild-type host cell. In some embodiments, the desired metabolic product is an alcohol (*e.g.*, ethanol).

[0114] In some embodiments, nucleic acid constructs comprising at least one further polynucleotide that is capable of conferring a desired phenotype to a fungal host cell comprise a polynucleotide encoding a protein known to impact the desired phenotype, wherein the polynucleotide is either native or heterologous to the fungal host cell. In some embodiments, this polynucleotide is operatively linked to its native promoter, or to a heterologous promoter (*i.e.*, a promoter that is not associated with the polynucleotide in the corresponding native gene). In some embodiments, at least one further polynucleotide is overexpressed. In some additional embodiments, the nucleic acid constructs comprise multiple copies of a least one polynucleotide. Suitable polynucleotides include, but are not limited to those that facilitate overexpression of proteins known to have an impact on the desired phenotype.

[0115] Exemplary recombinant polynucleotides that are capable of conferring a desired phenotype to a fungal host cell include, but are not limited to recombinant polynucleotides (either wild-type or mutated forms) that encode a xylose and/or hexose transporter, xylose reductase, at least one enzyme from the pentose phosphate pathway, at least one glycolytic enzyme (*i.e.*, from the glycolytic metabolic pathway, at least one ethanologenic enzyme, regulatory sequences that enhance expression of these sequences, and/or combinations thereof. Additional recombinant polynucleotides (either wild-type or mutated forms) that find use in the present invention include, but are not limited to those that encode additional proteins involved in the pentose phosphate, glycolysis, and ethanologenic pathways, used alone or in combination in various embodiments of the present invention.

[0116] In some embodiments, transporter proteins find use in the present invention. Exemplary transporters include, but are not limited to GXF1, SUT1 and At6g59250 from *Candida intermedia, Pichia stipitis* and *Arabidopsis thaliana*, respectively (*See e.g.*, Runquist *et al.*, Biotechnol. Biofuels, 3:5 [2010], incorporated herein by reference), as well as HXT4, HXT5, HXT7, GAL2, AGT1, GXF2 (*See e.g.*, Matsushika *et al.*, Appl. Microbiol. Biotechnol., 84:37-53 [2009], incorporated herein by reference). In some embodiments, overexpression of native *S. cerevisiae* transporters is desirable, particularly HXT5 and HXT7.

[0117] In some embodiments, additional recombinant polynucleotides find use, including but not limited to those that encode: xylulose reductase (XR); an enzyme from the pentose phosphate

pathway (*e.g.*, a ribulose-5-phosphate 3-epimerase (RPE1), ribose-5-phosphate ketol-isomerase (RKI1), transketolase (TKL1), transaldolase (TAL1), etc.); glycolytic enzyme(s) (*e.g.*, a hexokinase (HXK1/HXK2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase (PVK2), etc.); and/or at least one ethanologenic enzyme (*e.g.*, pyruvate decarboxylase, alcohol dehydrogenase, etc.).

[0118] In some embodiments of the present invention, regulatory polynucleotides find use. Exemplary regulatory polynucleotides include promoters, enhancers, terminators, and any other suitable regulatory element that functions to improve the expression of polynucleotides in a fungal host cell, particularly, a yeast host cell. These polynucleotides include, but are not limited to the regulatory elements described hereinabove.

[0119] The nucleic acid constructs described herein are useful for transforming fungal host cells to confer to these cells the property of xylose utilization.

Recombinant Fungal Host Cells

[0120] The present invention provides a recombinant fungal host cell comprising at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide provided herein. In some embodiments, the recombinant fungal host cell comprises at least one polynucleotide sequence that encodes a polypeptide capable of catalyzing the isomerization of D-xylose directly to D-xylulose. In some embodiments, the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; SEQ ID NO:3; and/or SEQ ID NO:5. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation. In all of these embodiments, the polypeptides exhibit the activity associated with their sequences (i.e., xylose isomerase, xylitol dehydrogenase, or xylulokinase).

[0121] In some embodiments, the recombinant fungal host cell comprises at least one polynucleotide sequence that encodes at least one polypeptide, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising 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 at least 100% identical to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; SEQ ID NO:4; and/or SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; SEQ ID NO:3; and/or SEQ ID NO:5. In some embodiments, the polypeptide(s) comprises at least one substitution and/or other mutation. In all of these embodiments, the polypeptides exhibit the activity associated with their sequences (i.e., xylose isomerase, xylitol dehydrogenase, or xylulokinase).

[0122] In some embodiments, the present invention provides a recombinant fungal host cell comprising and/or transformed with a nucleic acid construct of the present invention. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide is integrated into the host cell genome. In some embodiments, the xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotide are integrated into the host cell genome. Typically, the recombinant fungal host cell is a filamentous fungal or yeast host cell. More typically, the recombinant fungal host cell is a yeast host cell.

[0123] The present invention also provides methods for producing a recombinant fungal host cell, wherein the method comprises: (a) providing at least one nucleic acid construct of the present invention, wherein the nucleic acid construct comprises at least one xylose isomerase polynucleotide, at least one xylitol dehydrogenase polynucleotide, and/or at least one xylulokinase polynucleotide provided herein; and (b) transforming a fungal host cell with the nucleic acid construct to produce a recombinant fungal host cell. In some embodiments, the xylose isomerase polynucleotide sequence, xylitol dehydrogenase polynucleotide sequence, and/or xylulokinase polynucleotide sequence is integrated into the host cell genome.

[0124] The present invention further provides methods for producing a recombinant fungal host cell, wherein the method comprises: (a) providing at least one nucleic acid construct of the present invention, wherein the nucleic acid construct comprises at least one xylose isomerase polynucleotide, at least one xylitol dehydrogenase polynucleotide, and at least one xylulokinase polynucleotide provided herein; and (b) transforming a fungal host cell with the nucleic acid construct to produce a recombinant fungal host cell. In some embodiments, the xylose isomerase polynucleotide sequence, xylitol dehydrogenase polynucleotide sequence, and xylulokinase polynucleotide sequence is integrated into the host cell genome.

[0125] Introduction of the expression construct of the present invention into the host cell can be accomplished using any suitable method, including but not limited to calcium phosphate transfection, DEAE-dextran mediated transfection, electroporation, or any other suitable technique. Indeed, there are numerous methods known in the art and described in various standard reference texts.

[0126] In some embodiments of the present invention, the fungal host cells include yeast and filamentous fungal host cells. In some additional embodiments, the fungal host cell is a yeast cell. Exemplary yeast host cells useful in the present invention include, but are not limited to *Candida, Hansenula, Saccharomyces, Schizosaccharomyces, Pichia, Kluyveromyces,* and *Yarrowia.* In some embodiments of the invention, the yeast cell is *Hansenula polymorpha, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces diastaticus, Saccharomyces norbensis, Saccharomyces kluyveri, Schizosaccharomyces pombe, Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia kodamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia quercuum, Pichia pijperi, Pichia stipitis, Pichia methanolica, Pichia angusta, Kluyveromyces lactis, Candida albicans,* or *Yarrowia lipolytica.* In some embodiments, the yeast host cell is *Saccharomyces* species. In some additional embodiments, the yeast host cell is *Saccharomyces cerevisiae.* However, it is not intended that the present invention be limited to any particular genus and/or species of yeast cells.

[0127] Yeast strains that find use in the present invention include, but are not limited to those available from various yeast collections, such as Lallemand (e.g., Lallemand 6469, Lallemand LYCC 6391, Lallemand LYCC 6939, Lallemand LYCC 6469, Lallemand LYCC 6469; all from Lallemand, Inc., Montreal, Canada); ARS (NRRL) Collection, U.S. Department of Agriculture (e.g., NRRL Y-1528, and YB-1952); and ATCC (e.g., BY4741, which is also available from other sources).

[0128] Suitable fungal host cells include, but are not limited to, Ascomycota, Basidiomycota, Deuteromycota, Zygomycota, and Fungi Imperfecti. The filamentous fungal host cells of the present invention include, but are not limited to all filamentous forms of the subdivision Eumycotina and Oomycota. Filamentous fungi are characterized by a vegetative mycelium with a cell wall composed of chitin, cellulose and other complex polysaccharides. In some embodiments, the filamentous fungal host cells of the present invention are morphologically distinct from yeast.

[0129] In some embodiments, the filamentous fungal host cell is a cell of a species of *Achlya, Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Cephalosporium, Chrysosporium, Cochliobolus, Corynascus, Cryphonectria, Cryptococcus, Coprinus, Coriolus, Diplodia, Endothia, Fusarium, Gibberella, Gliocladium, Humicola, Hypocrea, Myceliophthora, Mucor, Neurospora, Penicillium, Podospora, Phlebia, Piromyces, Pyricularia, Rhizomucor, Rhizopus, Schizophyllum, Scytalidium, Sporotrichum, Talaromyces, Thermoascus, Thielavia, Trametes, Tolypocladium, Trichoderma, Verticillium, Volvariella,* or teleomorphs, or anamorphs, and synonyms, basonyms, and/or taxonomic equivalents thereof. However, it is not intended that the present invention be limited to any particular genus and/or species of filamentous fungal cells.

[0130] In some embodiments of the invention, the filamentous fungal host cell is of the *Aspergillus* species, *Ceriporiopsis* species, *Chrysosporium* species, *Corynascus* species, *Fusarium* species, *Humicola* species, *Neurospora* species, *Penicillium* species, *Tolypocladium* species, *Tramates*

species, or *Trichoderma* species. However, it is not intended that the present invention be limited to any particular genus and/or species of filamentous fungal cells.

[0131] Additionally, exemplary filamentous fungal host cells that find use in the present invention include, but are not limited to a filamentous fungal host cell of *Trichoderma* (*e.g., T. longibrachiatum, T. viride* [*e.g.*, ATCC 32098 and 32086], *T. reesei* [NRRL 15709, ATTC 13631, 56764, 56765, 56466, 56767, and RL-P37 and derivatives thereof; *See e.g.,* Sheir-Neiss *et al.,* Appl. Microbiol. Biotechnol., 20:46-53 [1984], incorporated herein by reference), *T. koningii*, and *T. harzianum*), as well as *Hypocrea jecorina*. The term "*Trichoderma*" refers to any fungal strain that was previously classified as *Trichoderma* or is currently classified as *Trichoderma*.

[0132] In some embodiments of the present invention, the filamentous fungal host cell is an *Aspergillus* species (*e.g., A. awamori, A. funigatus, A. japonicas, A. nidulans, A. niger, A. aculeatus, A. foetidus, A. oryzae, A. sojae,* or *A. kawachi* (*See e.g.*, Kelly and Hynes, EMBO J., 4:475479 [1985]; NRRL 3112, ATCC 11490, 22342, 44733, and 14331; Yelton *et al.,* Proc. Natl. Acad. Sci. USA, 81, 1480-1474 [1984]; Tilburn *et al.*, Gene 26,205-221 [1982]; and Johnston *et al.*, EMBO J., 4:1307-1311 [1985], all of which are incorporated herein by reference). In some embodiments of the invention, the filamentous fungal host cell is a *Fusarium* species (*e.g., F. bacterioides, F. cerealis, F. crookwellense, F. culmorum, F. graminaearum, F. graminum, F. oxysporum, F. rosium,* or *F. venenatum*). In some embodiments of the invention, the filamentous fungal host cell is of a *Neurospora* species (*e.g., N. crassa*; *See e.g.*, Case, *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 [1979]; US Pat. No. 4,486,553; and Kinsey and Rambosek, Mol. Cell. Biol., 4:117-122 [1984], all of which are incorporated herein by reference). In some embodiments of the invention, the filamentous fungal host cell is of a *Humicola* species (*e.g., H. insolens, H. grisea,* or *H. lanuginosa*). In some embodiments of the invention, the filamentous fungal host cell is a *Mucor* species (*e.g., M. miehei* or *M. circinelloides*). In some embodiments of the invention, the filamentous fungal host cell is a *Rhizopus* species (*e.g., R. oryzae* or *R. niveus*). In some embodiments of the invention, the filamentous fungal host cell is of a *Penicillium* species (*e.g., P. purpurogenum, P. chrysogenum,* or *P. verruculosum*). In some embodiments of the invention, the filamentous fungal host cell is a *Thielavia* species (*e.g., T. terrestris*). In some embodiments of the invention, the filamentous fungal host cell is a *Tolypocladium* species (*e.g., T. inflatum* or *T. geodes*). In some embodiments of the invention, the filamentous fungal host cell is a *Trametes* species (*e.g., T. villosa* or *T. versicolor*). In some embodiments of the invention, the filamentous fungal host cell is a *Chrysosporium* species, (*e.g., C. lucknowense, C. keratinophilum, C. tropicum, C. merdarium, C. inops, C. pannicola,* or *C. zonatum*). In some embodiments of the invention, the filamentous fungal host cell is of the *Myceliophthora* species, *e.g., M. thermophila*.

[0133] Strains that find use in the present invention include those that are readily accessible to the public from a number of culture collections, including but not limited to the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkutlturen GmbH (DSM),

Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL). Strains that find use in the present invention include those that are readily accessible to the public from any commercial source.

[0134] Recombinant fungal host cells of the present invention are capable of growth in a xylose-based culture medium (*i.e.*, a culture medium where xylose is the primary carbon source). In these xylose-based culture media, the carbon source comprises xylose. In some xylose-based culture media, the carbon source consists of xylose. In some embodiments, the recombinant fungal host cell is capable of faster growth in a xylose-based culture medium as compared to the corresponding wild-type fungal host cell. In some embodiments, the recombinant fungal host cell is capable of faster growth in a xylose-based culture medium as compared to wild-type *Saccharomyces cerevisiae*. In some embodiments, the recombinant fungal host cell is capable of growth at a rate of at least about 0.2 per hour (h^{-1}) in a xylose-based culture medium, while in some other embodiments, the growth rate is at least about 0.3 or 0.4 per hour (h^{-1}). Growth rate can be determined using any suitable method, including optical density, cell counting methods, etc. Indeed, there are various well known methods for determining cell growth that find use in the present invention. Exemplary xylose-based culture media include culture media that have been formulated to contain xylose (See, the Examples herein), as well as feedstock obtained from a cellulosic saccharification process and/or feedstock from a hemicellulose pre-treatment process (*i.e.*, a "hemicellulosic feedstock").

[0135] In some embodiments, recombinant fungal host cells of the present invention are also capable of fermenting xylose when provided with a xylose based culture medium. Typically, the recombinant fungal host cells described herein are capable of fermenting xylose at a faster rate compared to the corresponding wild-type fungal host cell. In some embodiments, the recombinant fungal host cells are capable of fermenting xylose at a rate of at least about 0.5 g/L/h, at least about 1g/L/h, at least about 2 g/L/h, at least about 3 g/L/h, at least about 4 g/L/h, at least about 5 g/L/h, at least about 6 g/L/h, at least about 7 g/L/h, at least about 8 g/L/h, at least about 9 g/L/h, or at least about 10 g/L/h. In some embodiments the recombinant fungal host cells are capable of fermenting xylose at a rate of at least about 0.1 g/g CDW/h, at least about 0.15 g/g CDW/h, at least about 2 g/g CDW/h, at least about 0.25 g/g CDW/h, at least about 0.3 g/g CDW/h, at least about 0.4 g/g CDW/h, at least about 0.5g/g CDW/h, at least about 0.6 g/g CDW/h, at least about 0.7 g/g CDW/h, at least about 0.75 g/g CDW/h, at least about 1 g/g CDW/h, at least about 1.25 g/g CDW/h, at least about 1.5 g/g CDW/h, at least about 1.75 g/g CDW/h, at least about 2 g/g CDW/h, at least about 2.25 g/g CDW/h, at least about 2.5 g/g CDW/h, at least about 2.75 g/g CDW/h, or at least about 3 g/g CDW/h. Exemplary xylose-based culture media include, but are not limited to culture media that have been formulated to contain xylose, as well as feedstock from cellulosic saccharification processes and/or feedstock from a hemicellulose pre-treatment process (*i.e.*, a "hemicellulosic feedstock").

[0136] In some embodiments, the fungal host cell is a wild-type fungal cell, while in some other embodiments, it is a mutated or otherwise altered or engineered form of a wild-type fungal cell (i.e., a

recombinant cell). In some embodiments, the fungal host cell (either wild-type or otherwise altered or engineered) comprises polynucleotides encoding xylose isomerase, xylitol dehydrogenase, xylulokinase, and one or more enzymes. In some embodiments, the additional enzyme is used in the pentose phosphate, glycolytic, and/or ethanologenic pathways. In some embodiments, the fungal host cell comprises polynucleotides encoding at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and all or some of the enzymes in the pentose phosphate, glycolytic, and ethanologenic pathways. In some embodiments, the fungal host cell comprises recombinant polynucleotides encoding enzymes that are heterologous to the fungal host cell (*i.e.*, not native to the fungal host cell). In some additional embodiments, the fungal host cell is engineered to comprise other metabolic pathways that utilize products/intermediates from the pentose phosphate, glycolytic, and/or ethanologenic pathways to produce other desirable products. For example, in some embodiments, the fungal host cell is engineered to comprise at least one metabolic pathway for the biosynthesis of a fatty alcohol or fatty acid (*See e.g.*, WO 2007/136762, incorporated herein by reference). In some embodiments, the fatty alcohol or fatty acid is a C8-C20 fatty acid or fatty alcohol. In some embodiments, the fungal host cell is altered or engineered to overexpress any one or more of the polynucleotides encoding the enzymes in one or more of these metabolic pathways.

[0137] In some embodiments, the recombinant fungal host cell of the present invention further comprises genetic modifications to the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide(s). In some embodiments, the recombinant host cell comprises at least one different recombinant polynucleotide that is capable of conferring a further desired phenotype to the fungal host cell. In some embodiments, the present invention provides a recombinant fungal host cell comprising at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotide and/or variant(s) thereof, and at least one recombinant polynucleotide that encodes a polypeptide that differs from the xylose isomerase, xylitol dehydrogenase, and xylulokinase or variant(s) thereof, wherein the recombinant polynucleotide imparts a desired phenotype to the fungal host cell. It is contemplated that in some embodiments, the recombinant polynucleotide that is capable of conferring a desired phenotype to the fungal host cell is introduced to the fungal host cell in the same nucleic construct as the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide(s), while in some other embodiments, the recombinant polynucleotide that is capable of conferring a desired phenotype to the fungal host cell is introduced to the fungal host cell in a different nucleic construct as the xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide(s). Nucleic acid constructs of the present invention comprising a xylose isomerase, xylitol dehydrogenase and/or xylulokinase polynucleotide(s) and at least one further recombinant polynucleotide capable of conferring a desired phenotype to the fungal host cell are described above.

[0138] In some embodiments, the recombinant polynucleotide that is capable of conferring a desired phenotype to the fungal host cell is a non-coding polynucleotide (*e.g.*, a regulatory polynucleotide), a coding polynucleotide, or a combination thereof. As described above, exemplary further desired

phenotypes include, but are not limited to increased transport of xylose into the host cell, increased xylose reductase activity, increased xylitol dehygrogenase activity, increased xylulokinase activity, increased xylose isomerase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to increased osmolarity, increased tolerance to organic acids, reduced production of by-products, and other like properties related to increasing flux through the pentose phosphate, glycolysis, and/or ethanologenic pathways to produce the desired metabolic product/intermediate at higher levels as compared to the corresponding wild-type host cell. In some embodiments, the desired metabolic product is an alcohol (*e.g.*, ethanol).

[0139] In some embodiments, recombinant fungal host cells comprising at least one further polynucleotide capable of conferring a desired phenotype to the fungal host cell comprise at least one polynucleotide encoding a protein known to impact the desired phenotype, wherein the polynucleotide is either native or heterologous to the fungal host cell. In some embodiments, the polynucleotide(s) is/are operatively linked to the native promoter(s), while in some other embodiments, the polynucleotide is operatively linked to a heterologous promoter (*i.e.*, one not associated with the polynucleotide in the corresponding native gene). In some embodiments, the polynucleotide is overexpressed. In some embodiments, the recombinant fungal host cell comprises multiple copies of the polynucleotide. Suitable polynucleotides include, but are not limited to those that facilitate overexpression of proteins known to have an impact on the desired phenotype. Therefore, in some embodiments, the fungal host cell is altered or engineered to overexpress one or more polynucleotides.

[0140] In some embodiments, recombinant polynucleotides that are capable of imparting a desired phenotype to a fungal host cell find use in the present invention include, but are not limited to recombinant polynucleotides that encode a xylose or hexose transporter, xylose reductase, at least one enzyme from the pentose phosphate pathway (*See e.g.*, Figure 2A), at least one glycolytic enzyme (*i.e.*, from the metabolic pathway of glycolysis; *See e.g.*, Figure 2B), ethanologenic enzyme(s) (*See e.g.*, Figure 2C), regulatory sequences associated with any of these sequences, and any combination thereof.

[0141] As indicated above, exemplary transporters that find use in the present invention include, but are not limited to GXF1, SUT1 and At6g59250 from *Candida intermedia, Pichia stipitis,* and *Arabidopsis thaliana*, respectively (*See e.g.*, Runquist *et al.,* 84:37-53 [2010], incorporated herein by reference), HXT4, HXT5, HXT7, GAL2, AGT1, and GXF2, (*See e.g.,* Matsushika *et al.*, Appl. Microbiol. Biotechnol.,84:37-53 [2009]). In some embodiments, overexpression of native *S. cerevisiae* transporters is desirable, particularly HXT5 and HXT7.

[0142] Also as indicated, above, recombinant polynucleotides suitable for use in the present invention include, but are not limited to those that encode: xylose reductase (XR); at least one enzyme from the pentose phosphate pathway (*e.g.*, a ribulose-5-phosphate 3-epimerase (RPE1), ribose-5-

phosphate ketol-isomerase (RKI1), transketolase (TKL1), transaldolase (TAL1), etc.); at least one glycolytic enzyme (*e.g.*, hexokinase (HXK1/HXK2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase (PVK2), etc.; and at least one ethanologenic enzyme (*e.g.*, pyruvate decarboxylase, alcohol dehydrogenase, etc.).

[0143] As indicated above, exemplary regulatory polynucleotides that find use in the present invention include promoters, enhancer, terminator, and other regulatory elements that function to improve the expression of polynucleotides in a fungal host cell, particularly, a yeast host cell, as described above.

[0144] In some embodiments, recombinant host cells of the present invention comprise one or more native genes deleted from its genome. In some embodiments, the deletion(s) cause removal or reduction of a biological activity that is otherwise exhibited by the fungal host cell. In some embodiments, the cumulative effect of the deletion(s) also leads to an improvement in a phenotype of the fungal host cell. Any suitable method for deleting gene finds use in the present invention. There are numerous methods well known in the art. For example, in some embodiments, recombinant host cells of the present invention have certain native genes deleted from the host genome in order to improve the utilization of pentose sugars (*e.g.*, xylose), increase transport of xylose into the host cell, increase xylose reductase activity, increase xylitol dehygrogenase activity, increase xylulokinase activity, increase xylose isomerase activity, increase flux through the pentose phosphate pathway, decrease sensitivity to catabolite repression, increase tolerance to ethanol/acetate, increase tolerance to increased osmolarity, increase tolerance to organic acids (low pH), reduce production of by-products, and other like properties related to increasing flux through the relevant pathways to produce ethanol and other desired metabolic products at higher levels, where comparison is made with respect to the corresponding host cell without the deletion(s). Genes targeted for deletion include, but are not limited to genes encoding enzymes in the pentose phosphate pathway, a glycolytic enzyme, and/or an ethanologenic enzyme, as well as any other gene, the deletion of which provides an advantage.

[0145] In some embodiments, other genes are targeted for deletion, including but not limited to those encoding aldose reductase (GRE3) (*See e.g.*, Matsushika *et al.*, Appl. Microbiol. Biotechnol., 84:37-53 [2009]), sorbitol dehydrogenases (SOR1/SOR2), glutamate dehydrogenase (GDH1), 6-phosphogluconate dehydrogenase (GND), glucose-5-phosphate dehydrogenase (ZWF1), and any enzyme for which its deletion is known in the art to improve the utilization of a pentose sugar, decrease by-product formation, and/or increase the ethanol yield of a fungal host cell. The genes encoding these enzymes in many fungi are known in the art. Those having ordinary skill in the art appreciate that additional genes encoding these and other enzymes of interest can be readily identified using various suitable techniques, such as by microarray analysis (*See e.g.*, Sedlak *et al.*, Yeast 21:671-684 [2004]), metabolic flux analysis (*See e.g.*, Sonderegger *et al.*, Appl. Environ. Microbiol., 70:2307-2317 [2004]), *in silico* modeling (*See e.g.*, Hjersted *et al.*, Biotechnol. Bioengineer. 97:1190-1204 [2007]), chemogenomics (*See e.g.*, Teixeira *et al.*, Appl. Environ. Microbiol., 75:5761-5772

[2009]), and other well known methods. Indeed, any suitable method finds use in the present invention.

[0146] In some embodiments, the host cells employed in the practice of the present invention are mutagenized and/or evolved to exhibit further desired phenotypes. For example, further improvements include, but are not limited to improvements in the utilization of pentose sugars (*e.g.*, xylose, arabinose, etc.), increased transport of xylose into the host cell, increased xylulose kinase activity, increased xylose reductase activity, increased xylitol dehydrogenase activity, increasesd xylose isomerase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol/acetate, increased tolerance to increased osmolarity, increased tolerance to organic acids (low pH), reduced production of by-products, and other like properties related to increasing flux through the pentose phosphate and glycolysis pathways to produce a desired metabolic product/intermediate at higher levels. In some embodiments, the desired metabolic product is an alcohol (*e.g.*, ethanol). In some embodiments, the host cells are mutagenized and/or evolved using known methods either prior to or after transformation with one or at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase polynucleotide. In some embodiments, the host cells are mutagenized and/or evolved using known methods either prior to or after transformation with one or at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polynucleotide. These methods include, but are not limited to classical mutagenesis, whole genome shuffling, evolutionary engineering methods, methods that employ screening and/or selection methods, and/or any combination of such well known methods.

[0147] Classical mutagenesis methods that find use in the present invention include, but are not limited to treatment of the host cell with a mutagen such as a chemical mutagen or irradiation exposure (*e.g.*, ultraviolet or gamma-irradiation). Whole genome shuffling methods involving, for example, recombination of genomic DNA between native genomic DNA sequences and/or variants thereof, can be facilitated by sexual mating, protoplast fusion methods and other methods well known in the art (*See e.g.*, WO 98/31837 and WO 2000/04190, incorporated herein by reference) also find use. In some embodiments, these methods are coupled with screening and/or selection methods to identify altered fungal host cells that exhibit the desired phenotype. For example, such methods find use in altering or engineering a fungal host cell to overexpress one or more desired polynucleotides. Indeed, any suitable method finds use in the present invention.

[0148] In some embodiments, evolutionary engineering is accomplished by prolonged cultivation and selection of strains under desired conditions through chemostat, turbidostat and/or batch cultures. Evolutionary engineering methods can be practiced under aerobic, microaerophilic or anaerobic conditions. Selection strategies can be optimized by varying culture conditions, for example, carbon source, nitrogen source, aeration, pH, temperature, etc. Methods for evolutionary engineering are well known in the art (*See e.g.*, Wisselink *et al.*, Appl. Environ. Microbiol., 75(4):907-914 [2009]; Kuyper *et al.*, FEMS Yeast Res., 5:399-409 [2005]; and Sauer, Adv. Biochem. Engineer. Biotechnol.,

73:129-169 [2001], all of which are incorporated herein by reference). Indeed, any suitable method finds use in the present invention.

[0149] In some embodiments of the present invention, the recombinant fungal host cell comprising a xylose isomerase, xylitol dehydrogenase and/or xylulokinase polynucleotide exhibits an improved phenotype relative to the corresponding fungal host cell without the xylose isomerase polynucleotide, xylitol dehydrogenase and/or xylulokinase polypeptide. In some embodiments, the recombinant fungal host cell comprises all three polynucleotides (i.e., xylose isomerase, xylitol dehydrogenase, and xylulokinase). In some embodiments, the improved phenotype comprises further improvement in the utilization of pentose sugars (*e.g.*, xylose, arabinose, etc.), increased transport of xylose into the host cell, increased xylulose kinase activity, increased xylose reductase activity, increased xylitol dehydrogenase activity, increasesd xylose isomerase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol/acetate, increased tolerance to increased osmolarity, increased tolerance to organic acids (low pH), and reduced production of by products, and/or other properties.

Enzyme Mixtures

[0150] In some embodiments, the present invention provides an enzyme mixture that comprises at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptide as provided herein. In some embodiments, the present invention provides an enzyme mixture that comprises at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase polypeptide as provided herein. In some embodiments, the enzyme mixture is cell-free (i.e., an enzyme mixture comprising enzymes that have been separated from cells), while in some alternative embodiments, the enzymes are not separated from the host cells that secrete at least one enzyme mixture component. Cell-free enzyme mixtures can be prepared by any of a variety of methodologies known in the art (e.g., filtration and/or centrifugation methodologies). In some embodiments, the enzyme mixtures are partially cell-free, substantially cell-free, or entirely cell-free.

[0151] In some embodiments, at least one xylose isomerase, xylitol dehydrogenase, xylulokinase, and any additional enzymes present in the enzyme mixture are secreted from a single genetically modified cell (e.g., a fungal host cell), while in some additional embodiments, at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and any additional enzymes present in the enzyme mixture are secreted from different microbes in combined or separate fermentations. Similarly, in some additional embodiments, at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and any additional enzymes present in the enzyme mixture are expressed individually or in sub-groups from different strains of different organisms and the enzymes are combined *in vitro* to make the enzyme mixture. It is also contemplated that the xylose isomerases and any additional enzymes in the enzyme mixture are expressed individually or in sub-groups from different strains of a single organism, and the enzymes combined to make the enzyme mixture. In some embodiments, all

of the enzymes are expressed from a single host organism, such as a genetically modified fungal cell. In some embodiments, the enzymes described in WO 2011/041594, WO 2011/066457, WO 2011/14363, WO 2011/150318, WO 2012/024698, WO 2012/027282, WO 2010/148148, WO 2012/024662, WO 2012/044868, WO 2012/061432, US Pat. Appln. Publ. No. 2012/0003703, US Pat. Appln. Publ. No. 2012/0083019, US Pat. Appln. Publ. No. 2012/0077216, US Pat. Appln. Publ. No. 2012/0045793, US Pat. Appln. Publ. No. 2012/0088271, US Pat. Appln. Publ. No. 2012/0107881, and/or US Pat. No. 8,143,050 (all of which are incorporated herein by reference), find use in the present invention.

[0152] In some embodiments, the enzyme mixture comprises at least one cellulase, selected from cellobiohydrolase (CBH), endoglucanase (EG), and/or beta-glucosidase (BGl) cellulases. Cellulase enzymes of the cellulase mixture work together in decrystallizing and hydrolyzing the cellulose from a biomass substrate to yield soluble sugars, such as but not limited to glucose (*See e.g.*, Brigham *et al. in* Wyman ([ed.], Handbook on Bioethanol, Taylor and Francis, Washington DC [1995], pp 119–141, incorporated herein by reference). In some embodiments, the cellobiohydrolase is *T. reesei* cellobiohydrolase II. In some embodiments, the endoglucanase comprises a catalytic domain derived from the catalytic domain of a *Streptomyces avermitilis* endoglucanase. In some embodiments, at least one cellulase is *Acidothermus cellulolyticus, Thermobifida fusca, Humicola grisea, Myceliophthora* (*e.g., M. thermophila*) and/or a *Chrysosporium* sp. cellulase. It is intended that the present invention encompass enzyme mixtures comprising any suitable cellulase obtained from any suitable source. It is not intended that the present invention be limited to any particular cellulase and/or cellulase source.

[0153] Cellulase mixtures for efficient enzymatic hydrolysis of cellulose are known (*See e.g.*, Viikari *et al.*, Adv. Biochem. Eng. Biotechnol.,108:121-45 [2007]; and US Pat. Publns. 2009/0061484; US 2008/0057541; and US 2009/0209009, each of which is incorporated herein by reference). In some embodiments, mixtures of purified naturally occurring or recombinant enzymes are combined with cellulosic feedstock or a product of cellulose hydrolysis. In some embodiments, one or more cell populations, each producing one or more naturally occurring or recombinant cellulases, are combined with cellulosic feedstock or a product of cellulose hydrolysis.

[0154] In some embodiments, at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptide(s) of the present invention is present in mixtures comprising at least one additional enzyme other than cellulases that degrade cellulose, hemicellulose, pectin, and/or lignocellulose. In some embodiments, the enzyme mixtures comprise at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptide(s) of the present invention, at least one cellulase, and at least one additional enzyme. In some embodiments, the enzymes comprise at least one xylanase, xylosidase, furanosidase, glucoronidase, esterase, acetylxylanesterase, feruloyl esterase, coumaroyl esterase, galactosidases, mannanases, mannosidases, pectinase, lyase, polygalacturonate lyase, galacturonase, pectin methyl esterase, galactanase, pectin acetyl esterase, pectin lyase, pectate lyase, rhamnosidase, polygalacturonate lyase, rhamnogalacturonanase, rhamnogalacturonan lyase,

galacturonohydrolase, arabinase, lignin-degrading enzyme, laccase, peroxidase, lipase, protease, amylase, expansin, expansin-like protein, cellulose integrating protein, scaffoldin, scaffoldin-like protein, cellulose-induced protein or modulating protein, and/or any additional enzyme of interest. It is intended that the present invention encompasses any enzyme combination and any enzyme concentration(s). Indeed, it is not intended that the present invention be limited to any particular enzyme combination(s) and/or enzyme concentrations, as those of skill in the art know to produce useful enzyme combinations and concentrations as needed for their particular uses.

[0155] A "hemicellulase" as used herein, refers to a polypeptide that can catalyze hydrolysis of hemicellulose into small polysaccharides such as oligosaccharides, or monomeric saccharides. Hemicellulloses include xylan, glucuonoxylan, arabinoxylan, glucomannan and xyloglucan. Hemicellulases include, for example, the following: endoxylanases, b-xylosidases, a-L-arabinofuranosidases, a-D-glucuronidases, feruloyl esterases, coumarolyl esterases, a-galactosidases, b-galactosidases, b-mannanases, and b-mannosidases. In some embodiments, the present invention provides enzyme mixtures that comprise at least one xylose isomerase, xylitol dehydrogenase, and/or xylulokinase polypeptide(s) of the present invention and one or more hemicellulases.

[0156] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one endoxylanase. Endoxylanases (EC 3.2.1.8) catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylans. This enzyme may also be referred to as endo-1,4-β-xylanase or 1,4-β-D-xylan xylanohydrolase. In some embodiments, an alternative is EC 3.2.1.136, a glucuronoarabinoxylan endoxylanase, an enzyme that is able to hydrolyze 1,4 xylosidic linkages in glucuronoarabinoxylans.

[0157] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one β-xylosidase. β-xylosidases (EC 3.2.1.37) catalyze the hydrolysis of 1,4-β-D-xylans, to remove successive D-xylose residues from the non-reducing termini. This enzyme may also be referred to as xylan 1,4-β-xylosidase, 1,4-β-D-xylan xylohydrolase, exo-1,4-β-xylosidase or xylobiase.

[0158] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one α-L-arabinofuranosidase . α-L-arabinofuranosidases (EC 3.2.1.55) catalyze the hydrolysis of terminal non-reducing alpha-L-arabinofuranoside residues in alpha-L-arabinosides. The enzyme acts on alpha-L-arabinofuranosides, alpha-L-arabinans containing (1,3)- and/or (1,5)-linkages, arabinoxylans, and arabinogalactans. Alpha-L-arabinofuranosidase is also known as arabinosidase, alpha-arabinosidase, alpha-L-arabinosidase, alpha-arabinofuranosidase, arabinofuranosidase, polysaccharide alpha-L-arabinofuranosidase, alpha-L-arabinofuranoside hydrolase, L-arabinosidase and alpha-L-arabinanase.

[0159] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one alpha-glucuronidase. Alpha-

glucuronidases (EC 3.2.1.139) catalyze the hydrolysis of an alpha-D-glucuronoside to D-glucuronate and an alcohol.

[0160] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one acetylxylanesterase. Acetylxylanesterases (EC 3.1.1.72) catalyze the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-napthyl acetate, and p-nitrophenyl acetate.

[0161] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one feruloyl esterase. Feruloyl esterases (EC 3.1.1.73) have 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase activity (EC 3.1.1.73) that catalyzes the hydrolysis of the 4-hydroxy-3-methoxycinnamoyl (feruloyl) group from an esterified sugar, which is usually arabinose in "natural" substrates, to produce ferulate (4-hydroxy-3-methoxycinnamate). Feruloyl esterase is also known as ferulic acid esterase, hydroxycinnamoyl esterase, FAE-III, cinnamoyl ester hydrolase, FAEA, cinnAE, FAE-I, or FAE-II.

[0162] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one coumaroyl esterase. Coumaroyl esterases (EC 3.1.1.73) catalyze a reaction of the form: coumaroyl-saccharide + H_2O = coumarate + saccharide. In some embodiments, the saccharide is an oligosaccharide or a polysaccharide. This enzyme may also be referred to as trans-4-coumaroyl esterase, trans-p-coumaroyl esterase, p-coumaroyl esterase or p-coumaric acid esterase. The enzyme also falls within EC 3.1.1.73 so may also be referred to as a feruloyl esterase.

[0163] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one alpha-galactosidase. Alpha-galactosidases (EC 3.2.1.22) catalyze the hydrolysis of terminal, non-reducing α-D-galactose residues in α-D- galactosides, including galactose oligosaccharides, galactomannans, galactans and arabinogalactans. This enzyme may also be referred to as melibiase.

[0164] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one beta-galactosidase. Beta-galactosidases (EC 3.2.1.23) catalyze the hydrolysis of terminal non-reducing β-D-galactose residues in β-D- galactosides. In some embodiments, the polypeptide is also capable of hydrolyzing α-L-arabinosides. This enzyme may also be referred to as exo-(1->4)-β-D-galactanase or lactase.

[0165] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one beta-mannanase. Beta-mannanases (EC 3.2.1.78) catalyze the random hydrolysis of 1,4-β-D-mannosidic linkages in mannans, galactomannans and glucomannans. This enzyme may also be referred to as mannan endo-1,4-β-mannosidase or endo-1,4-mannanase.

[0166] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one beta-mannosidase. Beta-

mannosidases (EC 3.2.1.25) catalyze the hydrolysis of terminal, non-reducing β-D-mannose residues in β-D- mannosides. This enzyme may also be referred to as mannanase or mannase.

[0167] In some embodiments one or more enzymes that degrade pectin are included in enzyme mixtures that comprise at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase of the present invention. A pectinase catalyzes the hydrolysis of pectin into smaller units such as oligosaccharide or monomeric saccharides. In some embodiments, the enzyme mixtures comprise any pectinase, for example an endo-polygalacturonase, a pectin methyl esterase, an endo-galactanase, a pectin acetyl esterase, an endo-pectin lyase, pectate lyase, alpha rhamnosidase, an exo-galacturonase, an exo-polygalacturonate lyase, a rhamnogalacturonan hydrolase, a rhamnogalacturonan lyase, a rhamnogalacturonan acetyl esterase, a rhamnogalacturonan galacturonohydrolase and/or a xylogalacturonase.

[0168] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one endo-polygalacturonase. Endo-polygalacturonases (EC 3.2.1.15) catalyze the random hydrolysis of 1,4-α-D-galactosiduronic linkages in pectate and other galacturonans. This enzyme may also be referred to as polygalacturonase pectin depolymerase, pectinase, endopolygalacturonase, pectolase, pectin hydrolase, pectin polygalacturonase, poly-α-1,4-galacturonide glycanohydrolase, endogalacturonase; endo-D-galacturonase or poly(1,4-α-D-galacturonide) glycanohydrolase.

[0169] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one pectin methyl esterase. Pectin methyl esterases (EC 3.1.1.11) catalyze the reaction: pectin + n H_2O = n methanol + pectate. The enzyme may also been known as pectin esterase, pectin demethoxylase, pectin methoxylase, pectin methylesterase, pectase, pectinoesterase or pectin pectylhydrolase.

[0170] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one endo-galactanase. Endo-galactanases (EC 3.2.1.89) catalyze the endohydrolysis of 1,4-β-D-galactosidic linkages in arabinogalactans. The enzyme may also be known as arabinogalactan endo-1,4-β-galactosidase, endo-1,4-β- galactanase, galactanase, arabinogalactanase or arabinogalactan 4-β-D- galactanohydrolase.

[0171] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one pectin acetyl esterase. Pectin acetyl esterases catalyze the deacetylation of the acetyl groups at the hydroxyl groups of GalUA residues of pectin.

[0172] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one endo-pectin lyase. Endo-pectin lyases (EC 4.2.2.10) catalyze the eliminative cleavage of (1→4)-α-D-galacturonan methyl ester to produce oligosaccharides with 4-deoxy-6-O-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends. The enzyme may also be known as pectin lyase, pectin trans-eliminase; endo-pectin

lyase, polymethylgalacturonic transeliminase, pectin methyltranseliminase, pectolyase, PL, PNL or PMGL or (1→4)-6-O-methyl-α-D-galacturonan lyase.

[0173] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one pectate lyase. Pectate lyases (EC 4.2.2.2) catalyze the eliminative cleavage of (1→4)-α-D-galacturonan to produce oligosaccharides with 4-deoxy-α-D-galact-4-enuronosyl groups at their non-reducing ends. The enzyme may also be known polygalacturonic transeliminase, pectic acid transeliminase, polygalacturonate lyase, endopectin methyltranseliminase, pectate transeliminase, endogalacturonate transeliminase, pectic acid lyase, pectic lyase, α-1,4-D-endopolygalacturonic acid lyase, PGA lyase, PPase-N, endo-α-1,4-polygalacturonic acid lyase, polygalacturonic acid lyase, pectin trans-eliminase, polygalacturonic acid trans-eliminase or (1→4)-α-D- galacturonan lyase.

[0174] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one alpha-rhamnosidase. Alpha-rhamnosidases (EC 3.2.1.40) catalyze the hydrolysis of terminal non-reducing α-L-rhamnose residues in α-L-rhamnosides or alternatively in rhamnogalacturonan. This enzyme may also be known as α-L-rhamnosidase T, α-L-rhamnosidase N or α-L-rhamnoside rhamnohydrolase.

[0175] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one exo-galacturonase. Exo-galacturonases (EC 3.2.1.82) hydrolyze pectic acid from the non-reducing end, releasing digalacturonate. The enzyme may also be known as exo-poly-α-galacturonosidase, exopolygalacturonosidase or exopolygalacturanosidase.

[0176] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one exo-galacturonase. Exo-galacturonases (EC 3.2.1.67) catalyze a reaction of the following type: (1,4-α-D-galacturonide)n + H2O = (1,4-α-D-galacturonide)n-i + D- galacturonate. The enzyme may also be known as galacturan 1,4-α-galacturonidase, exopolygalacturonase, poly(galacturonate) hydrolase, exo-D-galacturonase, exo-D- galacturonanase, exopoly-D-galacturonase or poly(1,4-α-D-galacturonide) galacturonohydrolase.

[0177] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one exopolygalacturonate lyase. Exopolygalacturonate lyases (EC 4.2.2.9) catalyze eliminative cleavage of 4-(4-deoxy-α-D-galact-4-enuronosyl)-D-galacturonate from the reducing end of pectate (*i.e.* de-esterified pectin). This enzyme may be known as pectate disaccharide-lyase, pectate exo-lyase, exopectic acid transeliminase, exopectate lyase, exopolygalacturonic acid-trans-eliminase, PATE, exo-PATE, exo-PGL or (1→4)-α-D-galacturonan reducing-end-disaccharide-lyase.

[0178] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one rhamnogalacturonanase.

Rhamnogalacturonanases hydrolyze the linkage between galactosyluronic acid and rhamnopyranosyl in an endo-fashion in strictly alternating rhamnogalacturonan structures, consisting of the disaccharide [(1,2-alpha-L-rhamnoyl-(1,4)-alpha-galactosyluronic acid].

[0179] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one rhamnogalacturonan lyase. Rhamnogalacturonan lyases cleave α-L-Rhap-(1→4)-α-D-GalpA linkages in an endo-fashion in rhamnogalacturonan by beta-elimination.

[0180] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one rhamnogalacturonan acetyl esterase. Rhamnogalacturonan acetyl esterases catalyze the deacetylation of the backbone of alternating rhamnose and galacturonic acid residues in rhamnogalacturonan.

[0181] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one rhamnogalacturonan galacturonohydrolase. Rhamnogalacturonan galacturonohydrolases hydrolyze galacturonic acid from the non-reducing end of strictly alternating rhamnogalacturonan structures in an exo-fashion. This enzyme may also be known as xylogalacturonan hydrolase.

[0182] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one endo-arabinase. Endo-arabinanases (EC 3.2.1.99) catalyze endohydrolysis of 1,5-α-arabinofuranosidic linkages in 1,5-arabinans. The enzyme may also be known as endo-arabinase, arabinan endo-1 ,5-α-L-arabinosidase, endo-1,5-α-L-arabinanase, endo-α-1,5-arabanase; endo-arabanase or 1,5-α-L-arabinan 1,5-α-L-arabinanohydrolase.

[0183] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one enzyme that participates in lignin degradation in an enzyme mixture. Enzymatic lignin depolymerization can be accomplished by lignin peroxidases, manganese peroxidases, laccases and cellobiose dehydrogenases (CDH), often working in synergy. These extracellular enzymes are often referred to as "lignin-modifying enzymes" or "LMEs." Three of these enzymes comprise two glycosylated heme-containing peroxidases, namely lignin peroxidase (LIP), Mn-dependent peroxidase (MNP), and a copper-containing phenoloxidase laccase (LCC).

[0184] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one laccase. Laccases are copper containing oxidase enzymes that are found in many plants, fungi and microorganisms. Laccases are enzymatically active on phenols and similar molecules and perform a one electron oxidation. Laccases can be polymeric and the enzymatically active form can be a dimer or trimer.

[0185] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one Mn-dependent peroxidase. The

enzymatic activity of Mn-dependent peroxidase (MnP) in is dependent on Mn^{2+}. Without being bound by theory, it has been suggested that the main role of this enzyme is to oxidize Mn^{2+} to Mn^{3+} (*See e.g.*, Glenn *et al.*, Arch. Biochem. Biophys., 251:688-696 [1986]). Subsequently, phenolic substrates are oxidized by the generated Mn^{3+}.

[0186] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one lignin peroxidase. Lignin peroxidase is an extracellular heme that catalyses the oxidative depolymerization of dilute solutions of polymeric lignin in vitro. Some of the substrates of LiP, most notably 3,4-dimethoxybenzyl alcohol (veratryl alcohol, VA), are active redox compounds that have been shown to act as redox mediators. VA is a secondary metabolite produced at the same time as LiP by ligninolytic cultures of *P. chrysosporium* and without being bound by theory, has been proposed to function as a physiological redox mediator in the LiP-catalyzed oxidation of lignin *in vivo* (*See e.g.*, Harvey, *et al.*, FEBS Lett., 195:242--246 [1986]).

[0187] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one protease and/or a lipase that participates in cellulose degradation.

[0188] As used herein, "protease" includes enzymes that hydrolyze peptide bonds (peptidases), as well as enzymes that hydrolyze bonds between peptides and other moieties, such as sugars (glycopeptidases). Many proteases are characterized under EC 3.4, and are suitable for use in the present invention. Some specific types of proteases include, cysteine proteases including pepsin, papain and serine proteases including chymotrypsins, carboxypeptidases and metalloendopeptidases.

[0189] As used herein, "lipase" includes enzymes that hydrolyze lipids, fatty acids, and acylglycerides, including phosphoglycerides, lipoproteins, diacylglycerols, and the like. In plants, lipids are used as structural components to limit water loss and pathogen infection. These lipids include waxes derived from fatty acids, as well as cutin and suberin.

[0190] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one expansin or expansin-like protein, such as a swollenin (*See e.g.*, Salheimo *et al.*, Eur. J. Biochem., 269:4202-4211 [2002]) or a swollenin-like protein. Expansins are implicated in loosening of the cell wall structure during plant cell growth. Expansins have been proposed to disrupt hydrogen bonding between cellulose and other cell wall polysaccharides without having hydrolytic activity. In this way, they are thought to allow the sliding of cellulose fibers and enlargement of the cell wall. Swollenin, an expansin-like protein contains an N-terminal Carbohydrate Binding Module Family 1 domain (CBD) and a C-terminal expansin-like domain. In some embodiments, an expansin-like protein or swollenin-like protein comprises one or both of such domains and/or disrupts the structure of cell walls (such as disrupting cellulose structure), optionally without producing detectable amounts of reducing sugars.

[0191] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one polypeptide product of a cellulose integrating protein, scaffoldin or a scaffoldin-like protein, for example CipA or CipC from *Clostridium thermocellum* or *Clostridium cellulolyticum* respectively. Scaffoldins and cellulose integrating proteins are multi-functional integrating subunits which may organize cellulolytic subunits into a multi-enzyme complex. This is accomplished by the interaction of two complementary classes of domain (*i.e.* a cohesion domain on scaffoldin and a dockerin domain on each enzymatic unit). The scaffoldin subunit also bears a cellulose-binding module that mediates attachment of the cellulosome to its substrate. A scaffoldin or cellulose integrating protein for the purposes of this invention may comprise one or both of such domains.

[0192] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one cellulose-induced protein or modulating protein, for example as encoded by cip1 or cip2 gene or similar genes from *Trichoderma reesei* (*See e.g.*, Foreman *et al.*, J. Biol. Chem., 278:31988-31997 [2003]), *M. thermophila*, and/or any other suitable organism.

[0193] In some additional embodiments, the present invention provides at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase, and at least one member of each of the classes of the polypeptides described above, several members of one polypeptide class, or any combination of these polypeptide classes to provide enzyme mixtures suitable for various uses.

Other Components of Xylose Isomerase/Xylitol Dehydrogenase/Xylulokinase Compositions

[0194] In some embodiments, xylose isomerase, xylitol dehydrogenase, and xylulokinase polypeptides of the present invention are used in combination with other optional ingredients such as at least one buffer, surfactant, and/or scouring agent. In some embodiments, at least one buffer is used with at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase polypeptides of the present invention (optionally combined with other enzymes) to maintain a desired pH within the solution in which the xylose isomerase, xylitol dehydrogenase, and xylulokinase are utilized. The exact concentration of buffer employed will depend on several factors which the skilled artisan can determine. Suitable buffers are well known in the art and any suitable buffer finds use in the present invention.

[0195] In some embodiments, at least one surfactant is used with at least one xylose isomerase, xylitol dehydrogenase, and xylulokinase of the present invention. Suitable surfactants include any surfactant compatible with the xylose isomerase(s), xylitol dehydrogenase(s), and xylulokinase(s), and any other enzymes present in the mixture. Exemplary surfactants include, but are not limited to anionic, non-ionic, and ampholytic surfactants. Suitable anionic surfactants include, but are not limited to, linear or branched alkylbenzenesulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefinsulfonates; alkanesulfonates,

and the like. Suitable counter-ions for anionic surfactants include, but are not limited to alkali metal ions, such as sodium and potassium; alkaline earth metal ions, such as calcium and magnesium; ammonium ion; and alkanolamines having from 1 to 3 alkanol groups of carbon number 2 or 3. Ampholytic surfactants suitable for use in the practice of the present invention include, but are not limited to surfactants such as quaternary ammonium salt sulfonates, betaine-type ampholytic surfactants, and the like. Suitable nonionic surfactants include, but are not limited to polyoxalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like. Mixtures of surfactants also find use in the present invention, as known in the art. Indeed, any suitable mixture of surfactants finds use in the present invention.

Fermentation

[0196] The present invention provides processes for producing fermentation products, wherein the method comprises: (a) providing the recombinant fungal cell of the present invention; (b) providing a fermentation medium comprising xylose; (c) contacting the fermentation medium with the recombinant fungal cell under conditions suitable for generating the fermentation product; and optionally (d) recovering the fermentation product. In some embodiments, the fermentation product is an alcohol (*e.g.*, ethanol, butanol, etc.), a fatty alcohol (*e.g.*, a C8-C20 fatty alcohol), a fatty acid (*e.g.*, a C8-C20 fatty acid), lactic acid, 3-hydroxypropionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, and/or a β-lactam (*e.g.*, cephalosporin). However, it is contemplated that other fermentation products will be produced using the methods of the present invention.

[0197] In some embodiments, the fermentation medium is feedstock from a cellulosic saccharification process and/or feedstock from a hemicellulose pre-treatment process. Such feedstocks include, but are not limited to carbohydrates (*e.g.*, lignocellulose, xylans, cellulose, starch, etc.), other sugars (*e.g.*, glucose, xylose, arabinose, etc.), and other compositions. Compositions of fermentation media suitable for the growth of yeast and filamentous fungi are well known in the art and there are various reference texts that provide recipes for these media. Any suitable medium finds use in the present invention.

[0198] Fermentation conditions suitable for generating desired fermentation products are well known in the art and any suitable method finds use in the present invention. In some embodiments, the fermentation process is carried out under aerobic or microaerophilic (*i.e.*, where the concentration of oxygen is less than that in air), or anaerobic conditions. In some embodiments, fermentation is conducted under anaerobic conditions (*i.e.*, no detectable oxygen), or less than about 5, about 2.5, or about 1 mmol/L/h oxygen. In the absence of oxygen, the NADH produced in glycolysis cannot be oxidized by oxidative phosphorylation. Under anaerobic conditions, pyruvate or a derivative thereof may be utilized by the host cell as an electron and hydrogen acceptor in order to generate NAD+. In

some embodiments of the present invention, when the fermentation process is carried out under anaerobic conditions, pyruvate is reduced to a fermentation product such as ethanol, butanol, lactic acid, 3-hydroxypropionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, and/or a β-lactam (*e.g.*, a cephalosporin).

[0199] In some embodiments, the fermentation process is run at a temperature that is optimal for the recombinant fungal cell. For example, in some embodiments, the fermentation process is performed at a temperature in the range of from about 20°C to about 42°C. In some embodiments, the process is carried out a temperature that is less than about 38°C, less than about 35°C, less than about 33°C, or less than about 38°C, but at least about 20°C, 22°C, or 25°C. However, in some embodiments, the temperature is much higher (*e.g.*, up to 100°C or greater). In some embodiments, recombinant host cells of the present invention are grown under batch or continuous fermentation conditions. Classical batch fermentation is a closed system, wherein the composition of the medium is set at the beginning of the fermentation and is not subject to artificial alterations during the fermentation. A variation of the batch system is a fed-batch fermentation, which also finds use in the present invention. In this variation, the substrate is added in increments as the fermentation progresses. Fed-batch systems are useful when catabolite repression is likely to inhibit the metabolism of the cells and/or where it is desirable to have limited amounts of substrate in the medium. Batch and fed-batch fermentations are common and well known in the art. Continuous fermentation is an open system where a defined fermentation generally maintains the culture at a constant high density where cells are primarily in log phase growth. Continuous fermentation systems strive to maintain steady state growth conditions. Methods for modulating nutrients and growth factors for continuous fermentation processes, as well as techniques for modulating nutrients and growth factors for continuous fermentation processes as well as techniques for maximizing the rate of product formation are well known in the art of industrial microbiology. It is intended that any suitable fermentation method will find use in the present invention.

[0200] The foregoing and other aspects of the invention may be better understood in connection with the following non-limiting examples.

EXPERIMENTAL

[0201] The present invention is described in further detail in the following Examples, which are not in any way intended to limit the scope of the invention as claimed.

[0202] In the experimental disclosure below, the following abbreviations apply: wrt (with regard to); pm (parts per million); M (molar); mM (millimolar), uM and µM (micromolar); nM (nanomolar); mol (moles); gm and g (gram); mg (milligrams); ug and µg (micrograms); L and l (liter); ml and mL (milliliter); cm (centimeters); mm (millimeters); um and µm (micrometers); sec. (seconds); min(s) (minute(s)); h(s) and hr(s) (hour(s)); U (units); MW (molecular weight); rpm (rotations per minute); °C (degrees Centigrade); DNA (deoxyribonucleic acid); RNA (ribonucleic acid); CDW (cell dry

weight); HPLC (high pressure liquid chromatography); HMF (hydroxymethylfurfural); ARS (ARS Culture Collection or NRRL Culture Collection, Peoria, IL); Lallemand (Lallemand Ethanol Technology, Milwaukee, WI); Dualsystems (Dualsystems Biotech AG, Basel, Switzerland); Megazyme (Megazyme International Ireland, Ltd., Wicklow, Ireland); Dasgip (Dasgip Biotools, LLC, Shrewsbury, MA); Difco (Difco Laboratories, BD Diagnostic Systems, Detroit, MI); PCRdiagnostics (PCRdiagnostics, E coli SRO, Slovak Republic); Agilent (Agilent Technologies, Inc., Santa Clara, CA); and Bio-Rad (Bio-Rad Laboratories, Hercules, CA).

[0203] The following media were used in the work described in the Examples below.

YPD:

- 10g/L Yeast Extract
- 20g/L Peptone
- 20g/L Dextrose

YP[5.5%]G (YPD with 5.5% glucose):

- 10g/L Yeast Extract (BD Difco Cat. No. 212750)
- 20g/L Peptone (BD Difco Cat. No. 211677)
- 55g/L Glucose

YP[5.5%]G[3.0%]X (YPD with 5.5% glucose and 3% xylose):

- 10g/L Yeast Extract (BD Difco Cat. No. 212750)
- 20g/L Peptone(BD Difco Cat. No. 211677)
- 55g/L Glucose
- 30g/L Xylose

SD Minimal Medium (also referred to as "synthetic medium," "minimal medium" and "defined minimal medium"):

- 6.7g/L Yeast Nitrogen Base, without amino acids, with ammonium sulfate (Sigma Y0626)
- 2g/L Synthetic Defined Mix (SD Complete, US Biological Cat. No. D9515)
- 3.06g/L Sodium phosphate, monobasic (Sigma S8282)
- 0.804g/L Sodium phosphate, dibasic (Sigma S7907)

Trace elements solution:

- 1.5 g/L EDTA
- 450 µg/L Zinc sulfate (Sigma 221376)
- 100 µg/L Manganese chloride (Sigma M3634)
- 30 µg/L Cobalt(II)chloride (Sigma 202185)
- 30 µg/L Copper(II)sulfate (Sigma C7631)
- 40 µg/L Disodiummolybdate (Sigma M1003)
- 450 µg/L Calcium chloride (Sigma C3881)
- 300 µg/L Iron(II)sulfate (Sigma F8048)
- 100 µg/L Boric acid (Sigma B0394)
- 10 µg/L Potassium iodide (US Bio P5000)

[0204] The following sequences find use in the present invention. SEQ ID NO:2 corresponds to a synthetic chimeric xylose isomerase polypeptide sequence ("Chimera 1") derived from various sources and SEQ ID NO:1 is the polynucleotide sequence encoding SEQ ID NO:2. SEQ ID NOS:7, 8, 9 and 10 are from *G. trabeum*, SEQ ID NOS:11 and 12 are from *Orpinomyces*, SEQ ID NOS:13 and 14 correspond to a xylose isomerase isolated from a cow's rumen, SEQ ID NOS:15 and 16 corresponds to a xylose isomerase isolated from a human gut, SEQ ID NO:17, 18, 19, and 20 correspond to xylose isomerases from *C. boidinii*, SEQ ID NOS:21 and 22 correspond to a xylose isomerase from *P. infestans*, and SEQ ID NOS:23 and 24 is from *B. hominus*; SEQ ID NO:4 corresponds to the xylitol dehydrogenase (XD) from *Pichia stipitis*, encoded by SEQ ID NO:3. SEQ ID NO:6 corresponds to the xylulokinase (XK) from *Saccharomyces cerevisiae* encoded by SEQ ID NO:5

Chimera 1:
ATGAAGAACTATTTCCCCAACGTCCCAGAAATTAAATACGAAGGTCCAAACTCCACAAA
TCCTTTCGCTTTTAAATATTATGATGCTAATAAAGTAGTCGCCGGTAAGACCATGAAGGA
GCATTTGAGATTCGCTCTATCCTGGTGGCACACTTTGTGTGCCGGTGGTGCTGATCCATTC
GGAGTAACTACTATGGACAGGACCTACGGTAACATTACCGACCCAATGGAACTAGCTAA
GGCCAAAGTTGATGCTGGTTTCGAACTGATGACTAAGCTGGGCATCGAGTTCTTCTGCTT
CCATGATGCCGACATTGCTCCAGAAGGTGACACCTTCGAAGAGTCCAAGAAAAATCTGT
TCGAGATTGTTGATTACATCAAGGAGAAGATGGACCAAACCGGCATCAAGTTGTTATGG
GGCACTGCTAACAACTTTAGTCACCCCAGGTTCATGCACGGTGCAGGAACTTCTCCTAGT
GCCGATGTTTTCGCTTATGCTGCTGCGAAAATAAAGAACGCTTTAGATGCGACCATCAAG
TTGGGCGGTAGAGGTTATGTCTTTTGGGGTGGTAGAGAAGGTTACGAGACCCTGCTGAAT
ACTAACATGGGCTTAGAACTGGACAACATGGCTAGGTTGATGAAGATGGCCGTTGAGTA
TGGTAGGTCTATTGGATTCAAAGGTGACTTCTACATCGAGCCTAAACCCAAGGAACCTAT
GAAGCACCAGTACGACTTCGACACTGCTACCGTATTAGGTTTTTTAAGGCAGTACGGGTT
GGATAAAGACTTCAAATTGAACATCGAAGCCAATCACGCCACACTAGCAGGACACTCAT
TCCAGCATGAGTTACGTATTGCTAGTATTAACGGTATGTTGGGTTCTGTTGATGCTAACC
AAGGTGACGTATTGTTAGGATGGGACACGGATCAATTCCCCACAAACATTTATGATACTA
CTATGTGTATGTATGAGGTCATTAAAGCCGGTGGTTTCACAAATGGCGGCCTGAACTTTG
ATGCTAAAGCTAGAAGAGGTTCATTCACGCCTGAAGATATTTTCTATTCTTACATTGCTG
GTATGGATGCTTTCGCGTTAGGGTTTAGAGCAGCTCTTAAATTGATTGAAGACGGTAGAA
TTGACAAGTTTGTGGCTGACAGGTATGCCTCTTGGAATACCGGTATTGGTGCAGATATTA
TTGCCGGAAAAGCCGATTTTGCATCATTGGAAAAATATGCTTTGGAGAAAGGAGAGGTT
ACCGCGTCATTGTCTTCTGGTAGACAAGAGATGCTGGAATCTATTGTCAACAACGTATTG
TTTAGTTTGTAA (SEQ ID NO:1)

MKNYFPNVPEIKYEGPNSTNPFAFKYYDANKVVAGKTMKEHLRFALSWWHTLCAGGADPF
GVTTMDRTYGNITDPMELAKAKVDAGFELMTKLGIEFFCFHDADIAPEGDTFEESKKNLFEIV
DYIKEKMDQTGIKLLWGTANNFSHPRFMHGAGTSPSADVFAYAAAKIKNALDATIKLGGRG
YVFWGGREGYETLLNTNMGLELDNMARLMKMAVEYGRSIGFKGDFYIEPKPKEPMKHQYD
FDTATVLGFLRQYGLDKDFKLNIEANHATLAGHSFQHELRIASINGMLGSVDANQGDVLLG
WDTDQFPTNIYDTTMCMYEVIKAGGFTNGGLNFDAKARRGSFTPEDIFYSYIAGMDAFALGF
RAALKLIEDGRIDKFVADRYASWNTGIGADIIAGKADFASLEKYALEKGEVTASLSSGRQEM
LESIVNNVLFSL (SEQ ID NO:2)

P. stipitis XD:

ATGACCGCTAATCCCTCTCTTGTTTTGAATAAGATTGACGACATTTCTTTTGAAACTTACG
ATGCTCCCGAAATTAGCGAACCCACAGACGTTTTAGTTCAAGTTAAAAAAAACTGGTATCT
GCGGTTCTGACATCCACTTCTACGCTCATGGAAGGATCGGCAACTTCGTCTTAACAAAGC
CAATGGTTCTGGGTCATGAAAGCGCGGGTACTGTTGTTCAAGTCGGTAAAGGTGTTACTT
CACTGAAGGTTGGTGATAACGTCGCAATCGAGCCCGGTATTCCATCTAGGTTCAGTGATG
AGTACAAATCTGGTCACTACAACCTGTGTCCACACATGGCATTTGCTGCTACTCCCAATT
CTAAAGAGGGTGAACCAAACCCACCAGGAACTCTATGTAAGTACTTCAAATCTCCAGAA
GACTTCCTGGTTAAGTTACCCGATCATGTTTCTTTGGAGTTGGGTGCTTTGGTCGAGCCAC
TATCTGTTGGGGTCCATGCTAGTAAATTAGGCTCCGTTGCATTTGGCGATTACGTTGCTGT
TTTTGGTGCTGGTCCAGTAGGATTACTGGCTGCCGCTGTCGCTAAGACATTTGGTGCCAA
GGGTGTGATTGTCGTTGATATATTTGACAACAAGCTGAAGATGGCCAAAGACATAGGTG
CCGCTACACATACCTTCAACTCCAAGACGGGAGGTAGTGAAGAATTGATCAAAGCCTTC
GGTGGTAATGTACCAAATGTTGTCTTGGAATGTACTGGGGCTGAACCATGTATTAAGCTA
GGTGTTGATGCCATCGCACCAGGTGGTAGATTCGTGCAAGTTGGTAATGCTGCTGGTCCC
GTGTCCTTTCCCATAACAGTGTTCGCTATGAAAGAACTTACTTTGTTTGGTTCATTTCGTT
ATGGTTTCAACGACTATAAGACAGCCGTGGGTATCTTTGATACTAACTACCAGAACGGTA
GAGAGAATGCTCCCATTGACTTTGAACAGCTTATCACGCACAGATACAAATTCAAAGAC
GCCATTGAAGCCTACGACCTAGTAAGAGCAGGTAAAGGGGCTGTCAAGTGTTTGATTGA
TGGTCCAGAATAA (SEQ ID NO:3)

MTANPSLVLNKIDDISFETYDAPEISEPTDVLVQVKKTGICGSDIHFYAHGRIGNFVLTKPMVL
GHESAGTVVQVGKGVTSLKVGDNVAIEPGIPSRFSDEYKSGHYNLCPHMAFAATPNSKEGEP
NPPGTLCKYFKSPEDFLVKLPDHVSLELGALVEPLSVGVHASKLGSVAFGDYVAVFGAGPVG
LLAAAVAKTFGAKGVIVVDIFDNKLKMAKDIGAATHTFNSKTGGSEELIKAFGGNVPNVVLE
CTGAEPCIKLGVDAIAPGGRFVQVGNAAGPVSFPITVFAMKELTLFGSFRYGFNDYKTAVGIF
DTNYQNGRENAPIDFEQLITHRYKFKDAIEAYDLVRAGKGAVKCLIDGPE (SEQ ID NO:4)

S.cerevisiae xylulokinase:

ATGCTGTGCTCCGTTATACAAAGGCAAACAAGAGAAGTATCCAACACTATGTCTTTAGAT
AGTTATTATCTAGGATTCGATTTAAGTACACAACAATTGAAATGTCTTGCTATAAACCAG
GATCTAAAGATCGTCCATTCCGAAACTGTCGAGTTCGAGAAGGACTTACCACATTATCAC
ACCAAGAAAGGCGTCTACATTCATGGTGACACCATCGAATGCCCAGTTGCTATGTGGTTA
GAAGCCCTGGATCTTGTCCTGTCCAAATATAGGGAGGCAAAGTTCCCACTGAACAAGGT
CATGGCTGTTTCCGGTTCTTGTCAGCAGCATGGCTCCGTCTACTGGTCATCACAGGCTGA
ATCTCTGTTAGAACAACTGAACAAGAAGCCAGAGAAGGACCTGTTACACTACGTCTCCTC
TGTTGCATTTGCCAGACAAACTGCTCCTAATTGGCAAGACCATTCCACTGCTAAACAATG
TCAGGAGTTCGAAGAGTGTATTGGTGGACCAGAGAAAATGGCCCAGTTAACTGGTTCCC
GTGCTCATTTCAGGTTCACAGGCCCACAAATCCTGAAGATTGCTCAGTTAGAACCAGAGG
CTTATGAAAAGACTAAGACCATCTCTTTGGTCTCTAATTTCTTAACTTCCATTCTGGTTGG
TCACTTGGTCGAACTGGAAGAAGCTGATGCGTGTGGTATGAACCTGTACGACATCCGTGA
GAGGAAGTTCTCTGACGAACTGCTGCATCTTATCGACTCCTCCTCTAAGGACAAGACCAT
CAGGCAGAAACTGATGAGGGCACCAATGAAGAACCTGATTGCCGGTACTATTTGCAAGT
ACTTCATCGAAAAGTATGGCTTCAACACCAACTGCAAAGTCTCCCCTATGACTGGCGATA
ACCTAGCCACCATTTGTAGCTTGCCCTTAAGAAAAAACGATGTTCTTGTGTCTTTGGGTA
CTTCCACAACCGTCTTGTTGGTTACCGACAAATATCACCCTTCACCAAACTACCACCTGTT
CATCCACCCGACGTTGCCTAACCACTACATGGGCATGATCTGCTACTGCAATGGCAGTTT
AGCAAGGGAAAGGATAAGGGACGAGTTGAACAAGGAGAGGGAGAACAACTACGAGAA
GACCAACGATTGGACCCTGTTCAACCAAGCTGTCCTGGATGATAGCGAATCCTCCGAGA
ATGAACTGGGCGTTTACTTTCCACTAGGCGAGATCGTTCCATCTGTCAAGGCCATCAACA
AGAGAGTAATCTTCAACCCCAAGACTGGCATGATCGAAAGGGAAGTCGCCAAGTTCAAG
GACAAGAGACATGACGCCAAGAACATCGTTGAATCTCAAGCCTTATCTTGCCGTGTTAGG

ATTTCTCCCCTACTAAGCGACTCCAATGCTTCTTCCCAGCAACGTTTGAACGAGGATACG
ATTGTTAAATTCGACTACGACGAGAGTCCATTGAGAGACTACTTGAACAAACGTCCTGAG
AGGACATTCTTTGTTGGTGGCGCATCCAAGAACGATGCTATTGTTAAGAAGTTTGCTCAG
GTCATAGGAGCAACCAAAGGTAACTTTCGTTTAGAAACTCCAAACTCATGCGCTTTAGGT
GGTTGCTACAAGGCTATGTGGTCTTTGTTGTATGATAGCAATAAAATCGCTGTTCCTTTCG
ACAAGTTCCTAAACGATAACTTCCCTTGGCACGTCATGGAATCCATCAGCGATGTAGACA
ACGAGAATTGGGATAGATACAATTCTAAAATAGTTCCCTTGTCTGAGTTAGAGAAGACCT
TGATTTAA (SEQ ID NO:5)

MLCSVIQRQTREVSNTMSLDSYYLGFDLSTQQLKCLAINQDLKIVHSETVEFEKDLPHYHTK
KGVYIHGDTIECPVAMWLEALDLVLSKYREAKFPLNKVMAVSGSCQQHGSVYWSSQAESLL
EQLNKKPEKDLLHYVSSVAFARQTAPNWQDHSTAKQCQEFEECIGGPEKMAQLTGSRAHFR
FTGPQILKIAQLEPEAYEKTKTISLVSNFLTSILVGHLVELEEADACGMNLYDIRERKFSDELL
HLIDSSSKDKTIRQKLMRAPMKNLIAGTICKYFIEKYGFNTNCKVSPMTGDNLATICSLPLRK
NDVLVSLGTSTTVLLVTDKYHPSPNYHLFIHPTLPNHYMGMICYCNGSLARERIRDELNKERE
NNYEKTNDWTLFNQAVLDDSESSENELGVYFPLGEIVPSVKAINKRVIFNPKTGMIEREVAKF
KDKRHDAKNIVESQALSCRVRISPLLSDSNASSQQRLNEDTIVKFDYDESPLRDYLNKRPERT
FFVGGASKNDAIVKKFAQVIGATKGNFRLETPNSCALGGCYKAMWSLLYDSNKIAVPFDKFL
NDNFPWHVMESISDVDNENWDRYNSKIVPLSELEKTLI (SEQ ID NO:6)

Sequences of Eukaryotic XI Genes:

EUK.1.XI:

ATGGATGGCGACTTAGATCCTAAAGAATATTTTCCTGAAATTCCTAAAATAAAGTATGAA
GGACCCGAATCTAAAAATCCAATGGCGTTTCATTATTATGATGCAGAAAAGGTGGTGAT
GGGTAAAAAGATGAAAGATTGGTTAAGGTTCGCAATGTGTTGGTGGCACACACTATGTG
CAGATGGCGCCGACCAATTTGGTGCTGGCACTAAGACATTCCCATGGAATGAAGGCTCA
GATCCAATCGCGATAGCTAAACAAAAAGTAGACGCTGGTTTTGAAATAATGCAAAAGCT
GGGGATTGAATACTACTGCTTCCACGATGTGGATCTAGTGTCTGAAGGGAATAGCGTTGA
AGAGTATGAGGCTAACTTAAAGCAGGTAGTTGCCTACTTGAAAGAAAAGCAACAACAAA
CGGGTATTAAACTGTTGTGGTCTACCGCGAATGTCTTCGGTAATAAGAGATACATGAATG
GTGCTTCTACAAACCCGGATTTCGATGTTGTCGCTAGAGCAATAGTGCAAATAAAAAATG
CTATGGATGCAGGGATTGAATTGGGAGCAGAAAATTATGTCTTCTGGGGAGGAAGAGAA
GGATATATGTCTTTATTGAATACTGACCAGAAGAGAGAAAAAGAACACATGGCTAGAAT
GCTTACTATGGCCAGAGATTACGCTAGAAGCAAGGGTTTTAAAGGTACGTTCTTAATCGA
ACCCAAACCCTGCGAGCCCTCTAAGCATCAGTATGATGTAGATACGGAAACTGTAATAG
GCTTCCTGAGGGCTCACAATCTAGACAAAGATTTCAAGGTAAATATCGAAGTCAACCAC
GCGACCCTTGCTGGACATACTTTTGAACACGAACTTGCGTGTGCAGTAGATGCGGGTATG
TTAGGTAGCATAGACGCAAATAGAGGTGATTATCAGAATGGATGGGATACCGATCAGTT
CCCTATTGACCAATACGAATTAGTACAAGCTTGGATGGAAATTATCAGGGGTGGCGGCTT
TACAACGGGCGGGACAAACTTTGATGCTAAAACCAGACGTAATTCTACTGACTTAGAGG
ATATTTTTATCGCTCATATAAGTGGTATGGACGCTATGGCACGTGCTTTGGAGAATGCCG
CAAAGTTACTGGAGGAATCTCCAATCCCCAAGATGAAGAAGGAAAGATACGCTTCATTC
GATTCTGGAATGGGTAAAGATTTCGAGGATGGTAAGTTAACGCTAGAGCAGGTTTATGA
GTACGGGAAGAAGAATGGTGAACCGAAGGATACTTCTGGAAAACAAGAACTTTACGAG
GCCATAGTAGCAATGTACGCTTAG (SEQ ID NO:7)

MDGDLDPKEYFPEIPKIKYEGPESKNPMAFHYYDAEKVVMGKKMKDWLRFAMCWWHTLC
ADGADQFGAGTKTFPWNEGSDPIAIAKQKVDAGFEIMQKLGIEYYCFHDVDLVSEGNSVEE
YEANLKQVVAYLKEKQQQTGIKLLWSTANVFGNKRYMNGASTNPDFDVVARAIVQIKNAM
DAGIELGAENYVFWGGREGYMSLLNTDQKREKEHMARMLTMARDYARSKGFKGTFLIEPK
PCEPSKHQYDVDTETVIGFLRAHNLDKDFKVNIEVNHATLAGHTFEHELACAVDAGMLGS

IDANRGDYQNGWDTDQFPIDQYELVQAWMEIIRGGGFTTGGTNFDAKTRRNSTDLEDIFI
AHISGMDAMARALENAAKLLEESPIPKMKKERYASFDSGMGKDFEDGKLTLEQVYEYGKK
NGEPKDTSGKQELYEAIVAMYA (SEQ ID NO:8)

EUK.2.XI:

ATGGATGGTGATTTAGATCCAAAAGAATATTTTCCAGAAATTCCTAAGATTAAATATGAA
GGTCCTGAATCCAAAAATCCAATGGCTTTTCATTATTATGATGCAGAAAAAGTTGTGATG
GGTAAAAAAAATGAAAGATTGGTTAAGATTCGCAATGTGTTGGTGGCACACATTATGTGC
AGATGGTGCAGACCAATTTGGTGCTGGTACTAAGACTTTTCCATGGAATGAAGGTTCTGA
TCCAATCGCTATCGCTAAACAAAAAGTTGATGCTGGTTTTGAAATTATGCAAAAGCTTGG
GATTGAATACTACTGTTTCCACGATGTCGATTTAGTCTCTGAAGGCAATTCCGTTGAAGA
ATATGAAGCTAACTTGAAGCAGGTTGTTGCATACTTGAAAGAAAAGCAACAACAAACCG
GTATTAAACTTTTGTGGTCTACAGCCAATGTATTCGGTAATAAGAGATACATGAATGGTG
CTTCTACAAACCCTGATTTTGATGTTGTTGCTAGAGCTATTGTCCAAATTAAAAATGCTAT
GGATGCTGGCATTGAATTGGGTGCTGAAAATTATGTATTTTGGGGTGGAAGAGAAGGTT
ATATGTCTTTGTTGAATACTGATCAAAAGAGAGAAAAAGAACACATGGCTAGAATGCTA
ACTATGGCAAGAGATTACGCTAGATCCAAGGGTTTTAAAGGTACTTTTTTGATAGAACCT
AAACCTTGCGAGCCTTCTAAACATCAGTATGATGTTGATACCGAAACTGTTATTGGTTTTT
TAAGGGCTCACAATTTAGACAAAGATTTTAAGGTTAATATCGAAGTTAATCACGCCACAT
TAGCTGGTCATACTTTTGAACACGAATTAGCCTGTGCTGTTGATGCCGGTATGTTGGGTTC
CATAGATGCTAATAGAGGTGATTATCAAAATGGTTGGGATACAGATCAATTCCCAATTGA
CCAATATGAATTGGTTCAAGCTTGGATGGAAATTATCAGAGGTGGAGGTTTTACAACGG
GTGGGACAAACTTTGATGCTAAAACAAGGAGAAATTCTACTGACTTAGAAGATATTTTTA
TCGCTCATATTTCAGGTATGGATGCTATGGCTAGGGCTTTGGAGAATGCCGCAAAATTAC
TGGAAGAATCTCCAATACCTAAGATGAAGAAGGAAAGATACGCTTCTTTTGATTCTGGTA
TGGGTAAAGATTTCGAAGATGGTAAGTTGACCTTAGAACAAGTTTATGAATACGGCAAA
AAAAATGGTGAACCTAAGGATACTTCTGGTAAACAAGAATTATACGAAGCAATTGTTGC
TATGTACGCTTAG (SEQ ID NO:9)

MDGDLDPKEYFPEIPKIKYEGPESKNPMAFHYYDAEKVVMGKKMKDWLRFAMCWWHTLC
ADGADQFGAGTKTFPWNEGSDPIAIAKQKVDAGFEIMQKLGIEYYCFHDVDLVSEGNSVEE
YEANLKQVVAYLKEKQQQTGIKLLWSTANVFGNKRYMNGASTNPDFDVVARAIVQIKNAM
DAGIELGAENYVFWGGREGYMSLLNTDQKREKEHMARMLTMARDYARSKGFKGTFLIEPK
PCEPSKHQYDVDTETVIGFLRAHNLDKDFKVNIEVNHATLAGHTFEHELACAVDAGMLGS
IDANRGDYQNGWDTDQFPIDQYELVQAWMEIIRGGGFTTGGTNFDAKTRRNSTDLEDIFI
AHISGMDAMARALENAAKLLEESPIPKMKKERYASFDSGMGKDFEDGKLTLEQVYEYGKK
NGEPKDTSGKQELYEAIVAMYA (SEQ ID NO:10)

EUK.3.XI:

ATGACCAAAGAATACTTCCCAACTATAGGTAAAATTAGATTTGAAGGTAAAGATAGCAA
GAATCCAATGGCATTTCACTACTACGATGCAGAAAAAGAAGTTATGGGCAAAAAGATGA
AAGATTGGCTGAGGTTCGCAATGGCTTGGTGGCACACACTTTGCGCAGATGGTGCAGATC
AATTTGGCGTCGGTACTAAATCATTCCCATGGAACGAAGGTACAGATCCTATTGCTATCG
CGAAACAGAAGGTTGATGCAGGATTCGAAATTATGACTAAATTGGGGATAGAGCATTAC
TGCTTTCATGATGTGGATCTTGTCTCCGAAGGGAACTCTATTGAAGAATATGAATCAAAC
TTGAAGCAAGTAGTTGCCTACTTGAAACAAAAGCAACAAGAAACGGGTATTAAACTGTT
GTGGTCTACCGCGAATGTCTTCGGTAATCCAAGATACATGAATGGTGCAAGCACTAACCC
TGACTTCGATGTGGTTGCACGTGCCATAGTTCAAATTAAGAACGCAATGGATGCAGGCAT
TGAGCTAGGTGCTGAAAATTATGTCTTCTGGGGAGGAAGAGAAGGATATATGTCTTTATT
GAATACTGACCAGAAGAGAGAAAAAGAACACATGGCTACTATGCTTACTATGGCCAGAG
ATTACGCTAGAAGCAAGGGTTTTAAAGGTACGTTCTTAATCGAACCCAAACCCATGGAG
CCCACGAAACATCAGTATGACGTTGATACAGAAACGGTCATTGGATTCCTGCGTGCCCAC

AACTTAGATAAAGATTTCAAAGTCAACATTGAAGTTAATCATGCGACCCTTGCTGGACAT
ACTTTTGAACACGAACTTGCGTGTGCAGTAGATGCGGGTATGTTAGGTAGCATAGACGCA
AATAGAGGTGATTATCAGAATGGATGGGATACCGATCAGTTCCCTATTGACCAATACGA
ATTAGTACAAGCTTGGATGGAAATTATCAGGGGTGGCGGCTTTGTAACGGGCGGGACAA
ACTTTGATGCTAAAACCAGACGTAATTCTACTGACTTAGAGGATATTATTATCGCTCATA
TAAGTGGTATGGACGCTATGGCACGTGCTTTGGAGAATGCCGCAAAGTTATTGCAAGAA
TCTCCATACTGTAATATGAAGAAGGAGAGATACGCTTCATTCGATTCTGGAATCGGTAAA
GATTTCGAGGATGGTAAGTTAACGCTAGAGCAGGTTTATGAGTACGGGAAGAAGAATGG
TGAACCGAAGGTCACTTCTGGAAAACAAGAACTTTACGAGGCCATAGTAGCAATGTACC
AATAA (SEQ ID NO:11)

MTKEYFPTIGKIRFEGKDSKNPMAFHYYDAEKEVMGKKMKDWLRFAMAWWHTLCADGAD
QFGVGTKSFPWNEGTDPIAIAKQKVDAGFEIMTKLGIEHYCFHDVDLVSEGNSIEEYESNL
KQVVAYLKQKQQETGIKLLWSTANVFGNPRYMNGASTNPDFDVVARAIVQIKNAMDAGIE
LGAENYVFWGGREGYMSLLNTDQKREKEHMATMLTMARDYARSKGFKGTFLIEPKPMEPT
KHQYDVDTETVIGFLRAHNLDKDFKVNIEVNHATLAGHTFEHELACAVDAGMLGSIDANR
GDYQNGWDTDQFPIDQYELVQAWMEIIRGGGFVTGGTNFDAKTRRNSTDLEDIIIAHISG
MDAMARALENAAKLLQESPYCNMKKERYASFDSGIGKDFEDGKLTLEQVYEYGKKNGEPK
VTSGKQELYEAIVAMYQ (SEQ ID NO:12)

EUK.4.XI:

ATGGCAAAAGAATATTTCCCGTTTACAGGTAAAATCCCATTCGAAGGTAAGGATTCAAA
AAATGTCATGGCTTTTCACTACTACGAGCCGGAAAAAGTGGTCATGGGCAAAAAAATGA
AAGATTGGTTGAAATTCGCAATGGCATGGTGGCACACTCTGGGAGGAGCAAGTGCAGAT
CAATTTGGCGGTCAAACCAGATCATACGAATGGGATAAGGCTGAATGTCCTGTTCAAAG
AGCGAAAGACAAGATGGACGCTGGATTCGAAATTATGGACAAGTTGGGTATTGAATATT
TTTGCTTTCATGACGTGGATCTTGTCGAGGAAGCGCCAACTATAGCTGAATACGAAGAAA
GAATGAAGGCTATTACTGATTATGCTCAAGAAAAGATGAAACAATTTCCGAATATTAAA
TTGCTGTGGGGTACTGCTAATGTGTTTGGCAACAAAAGATACGCTAACGGCGCTTCTACT
AACCCTGACTTTGATGTCGTTGCCAGAGCGATTGTACAAATAAAAAATAGCATTGATGCA
ACAATAAAGCTTGGCGGTACAAATTATGTCTTTTGGGGCGGAAGGGAAGGTTATATGTCT
TTATTGAATACTGACCAGAAGAGAGAAAAAGAACACATGGCTACTATGCTTGGTATGGC
CAGAGATTACGCTAGAGCCAAAGGTTTTAAAGGTACGTTCTTAATCGAACCCAAACCCAT
GGAGCCCTCTAAGCATCAGTATGATGTAGATACGGAAACTGTAATAGGCTTCCTGAAAG
CTCATGGTCTGGATAAGGACTTTAAAGTTAATATCGAGGTGAATCACGCAACTCTTGCTG
GTCATACATTCGAGCATGAACTTGCGTGTGCAGTAGATGCGGGTATGTTAGGTAGCATAG
ACGCAAATAGAGGTGATGCGCAGAATGGATGGGATACCGATCAGTTCCCTATTGACAAT
TTCGAATTAACACAAGCTATGTTAGAGATCATAAGGAATGGCGGCTTGGGAAATGGGGG
CACGAACTTTGACGCTAAAATTAGACGTAATTCTACTGACTTAGAGGATTTATTTATCGC
TCATATAAGTGGTATGGACGCTATGGCACGTGCTTTGATGAACGCCGCAGACATCTTGGA
AAACAGTGAATTGCCAGCCATGAAGAAGGCTAGATACGCTAGTTTTGATTCCGGTATCG
GCAAGGATTTCGAGGATGGTAAACTAACTTTTGAGCAGGTGTACGAATATGGTAAAAAA
GTCGAAGAACCAAAACAAACCTCTGGAAAGCAGGAGAAGTATGAAACAATTGTTGCTCT
ACACTGTAAGTAG (SEQ ID NO:13)

MAKEYFPFTGKIPFEGKDSKNVMAFHYYEPEKVVMGKKMKDWLKFAMAWWHTLGGASA
DQFGGQTRSYEWDKAECPVQRAKDKMDAGFEIMDKLGIEYFCFHDVDLVEEAPTIAEYEER
MKAITDYAQEKMKQFPNIKLLWGTANVFGNKRYANGASTNPDFDVVARAIVQIKNSIDATI
KLGGTNYVFWGGREGYMSLLNTDQKREKEHMATMLGMARDYARAKGFKGTFLIEPKPME
PSKHQYDVDTETVIGFLKAHGLDKDFKVNIEVNHATLAGHTFEHELACAVDAGMLGSIDAN
RGDAQNGWDTDQFPIDNFELTQAMLEIIRNGGLGNGGTNFDAKIRRNSTDLEDLFIAHISGMD
AMARALMNAADILENSELPAMKKARYASFDSGIGKDFEDGKLTFEQVYEYGKKVEEPKQTS
GKQEKYETIVALHCK (SEQ ID NO:14)

EUK.5.XI:

ATGGCGACTAAAGAGTACTTTCCAGGAATAGGAAAAATTAAATTCGAAGGTAAAGAGTC
CAAGAATCCAATGGCTTTCAGATATTACGATGCGGAAAAGGTAATAATGGGTAAAAAAA
TGAAGGATTGGCTGAAATTCTCCATGGCATGGTGGCACACTCTGTGTGCAGAAGGTGGA
GATCAATTTGGCGGCGGTACTAAACATTTCCCATGGAACGGTGATGCTGACAAGTTACAA
GCTGCGAAAAACAAGATGGACGCTGGATTCGAATTTATGCAGAAGATGGGTATTGAATA
TTATTGTTTCCATGATGTGGATTTATGTGACGAAGCGGACACTATTGAAGAATATGAAGC
TAACTTGAAGGCTATTGTTGCCTACGCTAAACAAAAGCAAGAAGAAACGGGTATTAAAC
TGTTGTGGGGCACTGCCAACGTGTTTGGCCACGCTAGATACATGAATGGCGCCGCAACTA
ACCCTGACTTTGATGTCGTTGCCAGAGCGGCTGTACAAATAAAAAATGCAATTGATGCAA
CAATAGAGCTTGGCGGTTCCAATTATGTCTTTTGGGGCGGAAGGGAAGGTTATATGTCTT
TATTGAATACTGACCAGAAGAGAGAAAAAGAACATTTGGCTCAAATGTTGACCATTGCT
AGAGACTATGCCCGTGCTAGAGGATTTAAGGGGACCTTCTTAATCGAACCCAAACCCAT
GGAGCCCACGAAACATCAGTATGACGTTGATACAGAAACGGTCGTTGGATTCCTGAAAG
CACATGGTCTGGATAAAGACTTTAAGGTCAACATTGAAGTTAATCATGCGACCCTTGCTG
GACATACTTTTGAACACGAACTTGCGGTCGCAGTAGATAACGGGATGTTGGGCTCAATTG
ATGCGAACAGAGGTGACTACCAGAATGGTTGGGATACCGATCAGTTTCCTATTGACAATT
ATGAGCTTACACAGGCCATGATGCAAATTATCAGAAACGGAGGTTTTGGTGACGGGGGT
ACAAATTTTGATGCTAAAACGAGGAGAAATTCAACCGACTTGGAAGATATTTTCATTGCC
CATATAGCAGGTATGGATGTTATGGCCAGGGCTTTGGAATCCGCAGCTAAATTGTTAGAG
GAATCTCCATATAAGAAAATGTTGGCTGACAGATACGCTTCATTCGATTCTGGAAAGGGT
AAAGAATTTGAGGAAGGTAAGTTAACGCTAGAGGACGTTGTTGCGTACGCTAAGGCTAA
TGGGGAGCCCAAACAAACTAGCGGCAAACAAGAATTGTATGAAGCTATTGTAAACATGT
ATTGCTAG (SEQ ID NO:15)

MATKEYFPGIGKIKFEGKESKNPMAFRYYDAEKVIMGKKMKDWLKFSMAWWHTLCAEGG
DQFGGGTKHFPWNGDADKLQAAKNKMDAGFEFMQKMGIEYYCFHDVDLCDEADTIEEYE
ANLKAIVAYAKQKQEETGIKLLWGTANVFGHARYMNGAATNPDFDVVARAAVQIKNAIDA
TIELGGSNYVFWGGREGYMSLLNTDQKREKEHLAQMLTIARDYARARGFKGTFLIEPKPMEP
TKHQYDVDTETVVGFLKAHGLDKDFKVNIEVNHATLAGHTFEHELAVAVDNGMLGSIDAN
RGDYQNGWDTDQFPIDNYELTQAMMQIIRNGGFGDGGTNFDAKTRRNSTDLEDIFIAHIAGM
DVMARALESAAKLLEESPYKKMLADRYASFDSGKGKEFEEGKLTLEDVVAYAKANGEPKQ
TSGKQELYEAIVNMYC (SEQ ID NO:16)

EUK.6.XI:

ATGCCAGCCTACTTTGACCAATTAGATAGAGTTAGATTCGAAGGTACACAAAGCACAAA
TCCATTGGCCTTTAGACATTACAACCCCGATGAAATAGTTCTAGGAAAAAGAATGGAAG
ACCACTTGAGATTTGCAGCCTGTTATTGGCATACCTTTTGTTGGAATGGTGCTGACATGTT
GGTATGGGTGCTTTCGACAGACCATGGCAACAACCCGGTGAAGCACTGGCTTTAGCAAA
ACGTAAGGCGGATGTCGCGTTTGAATTTTTCCATAAGTTGAATGTGCCATATTACTGTTTC
CACGATGTTGACGTTTCCCCAGAAGGAGCTAGCCTAAAAGAATATAAAAATAATTTCGC
ACAAATGGTCGATGTCTTAGCCGCTAAACAGGAACAGTCTGGTGTTAAGCTTCTGTGGGG
ACTGCTAATTGTTTTACCAATCCTCGTTATGGTGCAGGTGCGGCAACCAACCCAGACCCT
GAAGTTTTTAGCTGGGCAGCTACTCAAGTGGTTACTGCCATGGACGCTACTCATAAGTTG
GGTGGAGAAAATTACGTTTTATGGGGAGGTAGAGAAGGTTACGAAACCCTGTTGAATAC
GATTTAAGGCAGGAAAGAGAGCAAATTGGAAGGTTCATGCAGCTGGTTGTAGAGCATAA
ACACAAGATAGGCTTCCAGGGTACACTACTGATCGAACCTAAACCACAAGAACCGACCA
AGCATCAATATGATTACGACGCTGCGACAGTCTATGGATTCTTAAAGCAATTTGGTTTGG
AGAAGGAAATAAAGTTAAACATTGAAGCGAACTATGCAACCTTAGCAGGCCATTCTTTT
CACCATGGCATAGCAACAGCCATAGCATTAGGATTATTTGGTAGTGTTGATGCCAATAGG
GGGGACGCCCAGCTTGGTTGGGATACTGATCAGTTTCCAAATTCTGTTGAGGAAAACGCC

TTAGTCATGTACGAGATTCTAAAGGCTGGCGGATTTACTACAGGAGGTTTGAACTTTGAC
GCTAAGGTTAGGAGACAATCTACTGACAAATATGACTTGTTCTACGGTCATATCGGTGCT
ATGGATACAATGGCATTGTCTTTAAAAATAGCAGCTAGAATGATAGAGGCTGGAGGTTT
AGATCAAAGAGTCGCCAAAAGATATGCCGGTTGGAATGGTGAGTTGGGACAACAAATAT
TAAAAGGGCAGATGACGTTAACTGAAATAGCGCAGTACGCAGAACAACATAACCTTGCC
CCAGTTCATCAAAGCGGTCACCAGGAATTACTAGAGAATCTTGTTAATCATTACTTATTT
GATAAGTGA (SEQ ID NO:17)

MPAYFDQLDRVRFEGTQSTNPLAFRHYNPDEIVLGKRMEDHLRFAACYWHTFCWNGADMF
GMGAFDRPWQQPGEALALAKRKADVAFEFFHKLNVPYYCFHDVDVSPEGASLKEYKNNFA
QMVDVLAAKQEQSGVKLLWGTANCFTNPRYGAGAATNPDPEVFSWAATQVVTAMDATHK
LGGENYVLWGGREGYETLLNTDLRQEREQIGRFMQLVVEHKHKIGFQGTLLIEPKPQEPTK
HQYDYDAATVYGFLKQFGLEKEIKLNIEANYATLAGHSFHHGIATAIALGLFGSVDANRG
DAQLGWDTDQFPNSVEENALVMYEILKAGGFTTGGLNFDAKVRRQSTDKYDLFYGHIGAM
DTMALSLKIAARMIEAGGLDQRVAKRYAGWNGELGQQILKGQMTLTEIAQYAEQHNLAPV
HQSGHQELLENLVNHYLFDK (SEQ ID NO:18)

EUK.7.XI:

ATGCCCTATTTCCCAGGTGTTGAAAAAGTTAGATTCGAAGGCCCTGCAAGTACATCTGCA
CTAGCATTTAGACATTACGATGCGAATAAACTGATACTTGGAAAGCCAATGCGTGAACA
CTTGAGAATGGCAGCATGTTATTGGCATACCTTTGTTTGGCCCGGTGCTGACATGTTTGGT
ATGGGTACTTTCAAGAGACCATGGCAAAGAAGTGGAGAGCCAATGGAAGTAGCTATAGG
GAAGGCAGAAGCGGCTTTTGAGTTCTTCTCCAAACTAGGGATTGATTATTATAGCTTTCA
TGATACCGACGTTGCTCCTGAAGGATCTAGCCTAAAAGAATATAGGAATCATTTCGCACA
AATGGTCGATCATTTAGAAAGACATCAGGAACAGACCGGTATTAAGTTGCTTTGGGGGA
CAGCTAACTGCTTTTCTAATCCAAGGTTTGCCGCAGGCGCAGCTTCAAATCCTGATCCTG
AAGTTTTCGCATTTGCAGCTGCGCAAGTCTTCAGCGCAATGAATGCTACATTGAGATTGA
AAGGTGCTAATTATGTTTTGTGGGGTGGAAGAGAAGGTTATGAGACTTTGCTGAACACTG
ATTTAAAGAGAGAAAGGGAGCAATTGGGTCGTTTTATGCGTATGGTTGTAGAGCATAAA
CACAAGATAGGCTTCACTGGTGATTTGCTGATCGAACCTAAACCACAAGAACCGACCAA
GCATCAATATGATTACGACTCAGCGACAGTCTTTGGATTCTTACACGAATATGGTTTGGA
GCACGAAATAAAGGTTAACGTTGAAGCGAACCATGCAACCTTAGCAGGCCATTCTTTTCA
CCATGAAATAGCAACAGCCGTATCACTAGGTATATTTGGGAGTATTGATGCCAATAGGG
GGGACCCCCAGAATGGGTGGGACACAGACCAATTTCCAAATTCTGTAGAAGAGATGACT
TTAGCCACATACGAAATTCTAAAGGCTGGCGGATTTAAGAATGGAGGATACAACTTTGA
TTCTAAGGTTAGGAGACAATCTTTAGACGAAGTGGACTTGTTCCACGGTCATGTTGCAGC
TATGGATGTACTAGCCTTGGCTCTAGAGAGAGCTGCGGCTATGGTTCAAGATGACAGATT
GCAACAATTTAAAGATCAGAGATATGCAGGTTGGAGTCAGCCTTTAGGGCAGGCGGTAT
TAGCGGGCGAGTTCTCCTTAGAAAGTCTTGCCGAACATGCTTTTGCCAACGCATTAGACC
CTCAAGCTGTATCTGGGCGTCAAGAAATGCTTGAGGGTGTTGTTAACCGTTTTATTTAA
(SEQ ID NO:19)

MPYFPGVEKVRFEGPASTSALAFRHYDANKLILGKPMREHLRMAACYWHTFVWPGADMFG
MGTFKRPWQRSGEPMEVAIGKAEAAFEFFSKLGIDYYSFHDTDVAPEGSSLKEYRNHFAQ
MVDHLERHQEQTGIKLLWGTANCFSNPRFAAGAASNPDPEVFAFAAAQVFSAMNATLRLK
GANYVLWGGREGYETLLNTDLKREREQLGRFMRMVVEHKHKIGFTGDLLIEPKPQEPTKH
QYDYDSATVFGFLHEYGLEHEIKVNVEANHATLAGHSFHHEIATAVSLGIFGSIDANRGD
PQNGWDTDQFPNSVEEMTLATYEILKAGGFKNGGYNFDSKVRRQSLDEVDLFHGHVAAMD
VLALALERAAAMVQDDRLQQFKDQRYAGWSQPLGQAVLAGEFSLESLAEHAFANALDPQA
VSGRQEMLEGVVNRFI (SEQ ID NO:20)

EUK.8.XI:

ATGCAGCATCAAGTTAAAGAATATTTTCCAAACGTTCCAAAAATTACATTCGAGGGTCAA
AACGCTAAATCCGTACTTGCATACAGAGAATACAATGCTTCAGAAGTTATCATGGGAAA
GACTATGGAGGAATGGTGCAGGTTCGCAGTTTGTTACTGGCATACCTTCGGCAATTCTGG
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AGCGAATATATCTTCCAGGGAAAGATTGTTGGAAGCCGCAAAGTGCAAAGCTGACGCAG
CTTTTGAAACTTTTACGAAACTAGGTGTTAAGTATTATACCTTTCATGACGTGGATTTAAT
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TCTTGATAAACAAAATCAAACAGGGGTAAGATGCCTATGGGGTACTACCAATCTGTTCG
GACATAGACGTTTTATGAATGGTGCTTCTACTAATCCAGATATGAAAGTTTTTGCTCACG
CCGCAGCTAGAGTTAAGAAGGCTATGGAGATTACCCTGAAGTTGGGTGGACAAAACTTT
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ATTGGATCACATGGCAGCCTTCTTCAAGCTGGTGGTTGCATATAAAAAGGAACTGGGAG
CTACCTTCCAGTTTCTTGTTGAACCAAAGCCAAGGGAGCCCATGAAACACCAATATGATT
ACGATGCAGCTACGGTTGTCGCGTTCTTACACACTTATGGGTTACAAAACGACTTCAAAT
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MQHQVKEYFPNVPKITFEGQNAKSVLAYREYNASEVIMGKTMEEWCRFAVCYWHTFGNSG
SDPFGGETYTNRLWNESLERANISSRERLLEAAKCKADAAFETFTKLGVKYYTFHDVDLI
SEGANLEESQSLLDEISDYLLDKQNQTGVRCLWGTTNLFGHRRFMNGASTNPDMKVFAHA
AARVKKAMEITLKLGGQNFVFWGGREGFQSILNTDMKTELDHMAAFFKLVVAYKKELGAT
FQFLVEPKPREPMKHQYDYDAATVVAFLHTYGLQNDFKLNIEPNHTTLAGHDYEHDIYYA
ASYKMLGSVDCNTGDPLVGWDTDQFLMDEKKAVLVMKKIVEIGGLAPGGLNFDAKVRRES
TDLEDIFIAHIGSMDCFARGLRQAAKLLEKNELGELVKQRYASWKSTLGERIEQGQATLE
EVAAYAKESGEPDHVSGKQELAELMWSTVALATGIWQDHVTCSLTKNWC (SEQ ID NO:22)

EUK.9.XI:

ATGGAATATTTCCCAGGAATAAGTAATATCAAATATGAAGGGTCTGCGTCAATGAATGA
TCTAAGTTTTAAATGGTATAATGCTGAACAAGTTGTTTTAGGAAAGAAAATGAAGGACC
ATTTAAGATTTGCGGTTTGTTATTGGCATACCTTTTGCTACCAAGGTAATGATCAATTCGG
TGGACCTACTTTAAACAGACCGTGGTGCGGTGATGCAGATCCAATGGTTGAAGCTAAAA
AAAAGTGTGATGCGGCTTTTGAGTTCTTCACGAAACTTGGCGTAGAATACTATTGCTTCC
ATGATAGAGATATCGTTGCCGAGGGTGAGACTCTTGAAGAAACCAACAGGAGATTGGAT
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CCATGTGTTTGCTTTAGCAGCAGCGCAGGTAAAAAAGGCTATGGACATTACAAAAAAAC
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AAACATCAGTATGATTACGATGCACAAACTGTCATCGGTTTCCTGAGGAAATACGGTCTT
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TGAGCACGATATAGTTTTCGCTTGTAATGAGGGTATGCTAGGCTCAGTAGATGCGAACAC

TGGAGATACCCTTCTGGGCTGGGATACAGACCAGTTTCCAATGGACGTAAAGAAAGCCG
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ACGCTCACGTTAGGAGAGAATCTACCGATATGGAAGATAGATTTATTGCACACATTGGTG
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ATCAAGAAATGGTTGATAAAAGATACGAGTCCTACACAACCGGTATTGGGGCCAGGATC
GAAAATGGGGAGGCTACTTTTGAAGAGTGTGAAAAATACATTCTGGAAAATGGTAAACC
CGAACCTCAATCTGCTAAGCAAGAGAAATTCGAAATGTTATTAAATCATTACGTCTGA
(SEQ ID NO:23)

MEYFPGISNIKYEGSASMNDLSFKWYNAEQVVLGKKMKDHLRFAVCYWHTFCYQGNDQFG
GPTLNRPWCGDADPMVEAKKKCDAAFEFFTKLGVEYYCFHDRDIVAEGETLEETNRRLDE
ISDYMLEKQKQTGVKLLWGTANMFGDRVFMNGASTNPDAHVFALAAAQVKKAMDITKKL
GGENYVFWGGREGYQSILNSLPGKELDHMGQFMRMAVEYKKKIGATFQLLIEPKPREPTKH
QYDYDAQTVIGFLRKYGLEKDFKLNIEPNHTTLAGHDYEHDIVFACNEGMLGSVDANTGD
TLLGWDTDQFPMDVKKAVIVMYHIIRAGGLHSGGLNFDAHVRRESTDMEDRFIAHIGAMD
TFARALLIVEKIMNDKIYQEMVDKRYESYTTGIGARIENGEATFEECEKYILENGKPEPQ
SAKQEKFEMLLNHYV (SEQ ID NO:24)

EXAMPLE 1

Cloning the XI-XD-XK Pathway

[0205] The three genes, XI, XD and XK were cloned into the plasmid PLS0030112 (See, Figure 8) using restriction enzymes (*Bam*HI and *Nde*I for XI, *Spe*I and *Aat*II for XD, *Not*I and *Xho*I for XK) and ligated. Each gene was cloned under different promoter (TEF1, ADH1, and G3PD, respectively) resulting in the plasmid PLS0044980 (See, Figure 9). This plasmid was used to transform three yeast strains: Thermosacc® yeast (Lallemand), Thermosacc®-derived haploid progeny, and NRRL Y1528-derived haploid progeny. Transformation was performed based on the Sigma-Aldrich yeast transformation kit protocol and colonies were selected based on antibiotic resistance as observed by growth on YPD plates containing 200 ug/ml G418. In parallel experiments, these strains were also transformed with the control plasmids comprising the following gene combinations: XI only, XI+XD, XI+XK, XD+XK and a negative control (i.e., an empty vector).

EXAMPLE 2

Characterization of Strains Containing the XI-XD-XK Pathway

[0206] Colonies grown on YPD with 200 ug/ml G418 were picked after 48 hours of growth in five replicates into 96-well plates filled with 400ul of inoculation medium (YPD amended with 200 ug/ml G418, 1mM $MgSO_4$ and trace elements solution as provided above) and were grown for 24 hours at 30°C and 85% relative humidity, in a incubator-shaker at 2" throw and shaking speed of 250 RPM. After incubation, 80 ul of the culture were used to inoculate each well of a 96-deep well plate filled with 320 ul of YP[5.5%]G supplemented with 200 ug/ml G418, 1mM $MgSO_4$ and the trace elements solution provided above. Strains were grown for an additional 24 hours under the same conditions.

By the end of the propagation step, the plate was spun down (4000 RPM for ten minutes), and the cells were used to inoculate 400 ul YP[5.5%]G[3.0%]X amended with 200 ug/ml G418, 1mM $MgSO_4$ and trace elements solution as provided above). The fermentation process was performed in cap-mat sealed 96-well plates at 30 °C and 85% relative humidity, in incubator-shakers with 2" throw and a shaking speed of 100 RPM. At the end of 96 hours, the plate was spun down (4000 RPM for ten minutes) and the supernatant was filtered and analyzed for metabolites by a HPLC using standard methods known in the art. In some experiments, the residual xylose in supernatant was measured using a spectrophotometric assay (e.g. Megazyme xylose assay; CAT No. K-XYLOSE) performed according to the manufacturer's protocol. The improvement in performance for xylose utilization was calculated based on a comparison with the performance of a control strain that was only transformed with the antibiotic marker.

[0207] Figure 3 provides a graph showing the fermentation results. Fermentation analysis indicated a significant improvement in xylose consumption and ethanol production for all three strains comprising the XI-XD-XK pathways compared with the control strains comprising the XI gene only. While strains comprising XI in combination with XD or XK alone did not result in xylose utilization improvement, strains with the combined XI-XD-XK pathway achieved 2-3x fold higher xylose consumption than the corresponding parent strains comprising only XI. Moreover, these strains demonstrated lower xylitol (XOH) production, possibly due to the combined activity of XD and XK genes resulting in an increased in flux towards the pentose phosphate pathway (it is not intended that the present invention be limited to any specific mechanism and/or theory).

[0208] Figure 4 provides a graph showing the fold improvement in xylose consumption by different strains tested under several fermentation conditions. Xylose consumption by strains comprising the XI-XD-XK pathway were compared to those only comprising the XI gene. Xylose consumption was calculated over the consumption measured from a reference strain comprising the empty plasmid. In this Figure, "YPD5.5X3" refers to YP[5.5%]G[3.0%]X medium, "YPD5.5X5.5" is YP[5.5%]G medium with 5.5% xylose, and "SD5.5X3" is SD minimal medium with 5.5% glucose and 3.0% xylose. The coefficients of variation were between 2-6%. As shown in Figure 4, strains harboring the XI-XD-XK pathway maintained improved performance as observed by higher xylose consumption and corresponding ethanol production under a number of conditions, such as a fermentation time of 72 hours instead of 96 hours, xylose concentration of 5.5% instead of 3%, and minimal SD based media instead of YPD based media. The relative improvement was also observed when scaled up to 25 ml.

[0209] Figure 5 provides a time course analysis of 25 ml fermentation of haploid strains comprising the empty plasmid (i.e., the negative control), XI-XD-XK pathway, or XI gene only. Fermentation was performed in 25 ml scintillation vials in YPD5.5X3 (YPD medium containing 5.5% glucose and 3% xylose). Automated samples analysis demonstrated higher xylose consumption rate, higher

ethanol (EtOH) production, and lower xylitol (XOH) production by strains comprising the XI-XD-XK pathway compared with strain comprising only the XI gene.

EXAMPLE 3

Growth Rate Evaluation of XI-XD-XK Strains in Xylose Minimal Media

[0210] Strains NRRL-Y1528 and a Thermosacc®-derived haploid yeast strain comprising the XI-XD-XK pathway and control strains harboring only the XI gene were grown in defined mineral xylose medium in order to compare their specific growth rate on xylose. Samples were taken in order to measure the level of biomass and thus calculate the specific rate of growth.

[0211] Glycerol stocks containing the NRRL-Y1528 and Thermosacc®-derived haploid yeast strains prepared in Example 1 were inoculated into 5ml of SD minimal medium containing 2% glucose. Cells were grown for 24 hours in an incubator at 30 °C with shaking at 250 rpm. After incubation, 0.5 mls of this culture were then diluted into 50 ml of SD minimal medium containing 2% xylose as the only carbon source in a 250 ml shake flask. The cultures were allowed to grow at 30 °C with shaking at 160 rpm. Samples from the culture were removed for optical density measurements at 600 nm once or twice per day. Once the optical density of the culture reached a value above 5 it was diluted 10-fold with minimal medium containing 2% xylose as the only carbon source. The process was continued for 150 hours. During this time, the XI-only strains did not grow significantly, whereas the XI-XD-XK strains grew at a growth rate of 0.08 to 0.09 hr^{-1}.

[0212] In order to calculate the specific growth rate, a plot of the natural log of biomass concentration versus time was used to generate a linear correlation. The slope of this correlation yielded the specific growth rate for the strain under the specified conditions.

Genes	Growth Rate hr^{-1}	
	NRRL Y-1528 Strains	**Thermosacc®-Derived Haploid Strains**
XI only	No growth	0.01
XI-XD-XK	0.08	0.09
Fold improvement over XI-only strain	N/A	9

EXAMPLE 4

XI-XD-XK Integration at rDNA Locus

[0213] Integration of the genes encoding the XI-XD-XK pathway at the rDNA locus allows for stable co-expression of pathway components in yeast without continuous selection for the plasmid. Plasmid PLS0047984 is constructed by subcloning 5' and 3' homologous sequences from the *RDN* 1 locus using standard yeast recombination cloning techniques. PLS0047984 is linearized by digestion

with *Sma*I and *Pst*I to generate a linear fragment suitable for integrative transformation of target strains. Following outgrowth, selection for frequent recombination events is carried out on YPD containing 200 ug/ml G418 selective plates. PCR on the emergent colonies is performed to verify the presence of the XI gene following transformation.

EXAMPLE 5

Evaluation of XI Gene Diversity

[0214] Nine diverse copies of XI genes from eukaryotic origin were reconstructed from cDNA sequences or found in DNA sequences translated BLAT (See e.g., Kent, Genome Res. 12:656-664 [2002]) or BLAST searches. The sequences were harvested, codon optimized (in one case, the same gene was subjected to two alternate codon optimizations), synthesized with *Bam*HI and Kozak sequences upstream of their start codons and an *Nde*I sequence downstream of their stop codons. Sequences were subcloned into the *Bam*HI and *Nde*I sites of PLS030112, under the control of the *S. cerevisiae* TEF1 promoter. These plasmids were transformed into a yeast expression strain that does not encode any other exogenous genes for xylose utilization.

[0215] Colonies grown on YPD with 200 ug/ml G418 were picked after 48 hours of growth in five replicates into 96-well plate filled with 400 ul of (YPD containing 200 ug/ml G418, 1mM MgSO4 and the trace elements solution provided above) and were grown for 24 hours at 30 °C and 85% relative humidity, in a incubator-shaker at 2" throw and shaking speed of 250 RPM. After incubation, 80 ul of the culture were used to inoculate 96-deep well plate filled with 320 ul of YP[5.5%]G amended with 200 ug/ml G418, 1mM $MgSO_4$ and Trace elements solution. Strains were grown for an additional 24 hours under the same conditions. At the end of the propagation step, the plate was spun down (4000 RPM for ten minutes), and the cells were used to inoculate 400 ul of fermentation medium (YP[5.5%]G[3.0%]X amended with 200 ug/ml G418, 1mM MgSO4 and Trace elements solution). The fermentation process was performed in cap-mat sealed 96-well plates at 30 °C degree and 85% relative humidity, in an incubator-shaker 2" throw and shaking speed of 100 RPM. At the end of 72 hours, the plate was spun down (4000 RPM for ten minutes) and the supernatant was analyzed for residual xylose in the supernatant by using a spectrophotometric assay (e.g., Megazyme xylose assay; CAT No. K-XYLOSE) performed according to the manufacturer's protocol. Improvement in xylose utilization was determined by comparing the performance of the XI-transformed strain with that of a control strain that was transformed with only the antibiotic marker and a background strain comprising only the original XI gene.

[0216] Four of the nine strains (i.e., comprising one of the new XI sequences) consumed significantly more xylose than the negative control. The best strains were EUK.3 (*Orpinomyces* XI), EUK.4 (XI

obtained from cow rumen), EUK.5 (XI obtained from human gut), and EUK.9 (*B. hominis* XI). Figure 7 provides a graph showing the residual xylose as measured after fermentation of the control and transformed NRRL Y1528-derived haploid strains described in Example 1.

[0217] While particular embodiments of the present invention have been illustrated and described, it will be apparent to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the present invention. Therefore, it is intended that the present invention encompass all such changes and modifications with the scope of the present invention.

[0218] The present invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part(s) of the invention. The invention described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is/are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation. There is no intention that in the use of such terms and expressions, of excluding any equivalents of the features described and/or shown or portions thereof, but it is recognized that various modifications are possible within the scope of the claimed invention. Thus, it should be understood that although the present invention has been specifically disclosed by some preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be utilized by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

CLAIMS

We claim:

1. A recombinant fungal host cell comprising at least one nucleic acid construct, wherein said nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase, and/or at least one polynucleotide encoding a xylitol dehydrogenase, and/or at least one polynucleotide encoding a xylulokinase.

2. The recombinant fungal host cell of Claim 1, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic or prokaryotic enzymes.

3. The recombinant fungal host cell of Claim 2, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic enzymes.

4. The recombinant fungal host cell of any of Claims 1-3, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are fungal enzymes.

5. The recombinant fungal host cell of any of Claims 1-4, wherein said nucleic acid construct further comprises at least one genetic element that facilitates stable integration into a fungal host genome.

6. The recombinant fungal host cell of Claim 5, wherein said genetic element facilitates integration into a fungal host genome by homologous recombination.

7. The recombinant fungal host cell of Claim 5 and/or 6, wherein said genetic element comprises a fungal origin of replication.

8. The recombinant fungal host cell of Claim 7, wherein the fungal origin of replication is a yeast origin of replication.

9. The recombinant fungal host cell of any of Claims 1-8, wherein at least one of said polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell.

10. The recombinant fungal host cell of Claim 9, wherein said promoter sequence is a fungal promoter sequence.

11. The recombinant fungal host cell of Claim 9 and/or 10, wherein said fungal promoter sequence is a yeast promoter sequence.

12. The recombinant fungal host cell of any of Claims 1-11, wherein said polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell.

13. The recombinant fungal host cell of any of Claims 1-11, wherein said polynucleotide sequence contains codons optimized for expression in a yeast cell.

14. The recombinant fungal host cell of any of Claims 1-13, wherein said at least one polynucleotide is integrated into the host cell genome.

15. The recombinant fungal host cell of any of Claims 1-14, wherein the host cell has had one or more native genes deleted from its genome.

16. The recombinant fungal host cell of Claim 15, wherein the deletion of said one or more native gene results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced production of by products, wherein comparison is made with respect to the corresponding host cell without the deletion(s).

17. The recombinant fungal host cell of any of Claims 1-16, wherein the host cell is altered to overexpress one or more polynucleotides.

18. The recombinant fungal host cell of Claim 17, wherein overexpression results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced product of by products, wherein comparison is made to the corresponding unaltered host cell.

19. The recombinant host cell of any of Claims 1-18, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

20. The recombinant host cell of any of Claims 1-19, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, % 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 at least

100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

21. The recombinant fungal host cell of any of Claims 1-20, wherein said nucleic acid construct comprising at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3; and SEQ ID NO:5.

22. The recombinant fungal host cell of any of Claims 1-21, wherein said host cell is a yeast cell.

23. The recombinant fungal host cell of Claim 22, wherein said host cell is *Saccharomyces cerevisiae*.

24. A recombinant fungal host cell comprising at least one nucleic acid construct, wherein said nucleic acid construct comprises at least one polynucleotide encoding a xylose isomerase.

25. The recombinant fungal host cell of Claim 24, wherein said xylose isomerase is a eukaryotic or prokaryotic enzyme.

26. The recombinant fungal host cell of Claim 24 and/or 25, wherein said xylose isomerase is a eukaryotic enzyme.

27. The recombinant fungal host cell of any of Claims 24-26, wherein said xylose isomerase is a *G. trabeum* xylose isomerase, an *Orpinomyces* xylose isomerase, a xylose isomerase

obtained from a bovine rumen, a xylose isomerase obtained from a human gut, a *C. boidinii* xylose isomerase, *P. infestans* xylose isomerase, or *B. hominis* xylose isomerase.

28. The recombinant fungal host cell of any of Claims 24-27, wherein said nucleic acid construct further comprises at least one genetic element that facilitates stable integration into a fungal host genome.

29. The recombinant fungal host cell of Claim 28, wherein said genetic element facilitates integration into a fungal host genome by homologous recombination.

30. The recombinant fungal host cell of Claim 28 and/or 29, wherein said genetic element comprises a fungal origin of replication.

31. The recombinant fungal host cell of Claim 30, wherein the fungal origin of replication is a yeast origin of replication.

32. The recombinant fungal host cell of any of Claims 24-31, wherein at least one of said polynucleotide sequences is operatively linked to a promoter sequence that is functional in a fungal cell.

33. The recombinant fungal host cell of Claim 32, wherein said promoter sequence is a fungal promoter sequence.

34. The recombinant fungal host cell of Claim 32 and/or 33, wherein said fungal promoter sequence is a yeast promoter sequence.

35. The recombinant fungal host cell of any of Claims 24-34, wherein said polynucleotide sequence is operatively linked to a transcription termination sequence that is functional in a fungal cell.

36. The recombinant fungal host cell of any of Claims 24-35, wherein said polynucleotide sequence contains codons optimized for expression in a yeast cell.

37. The recombinant fungal host cell of any of Claims 24-36, wherein said at least one polynucleotide is integrated into the host cell genome.

38. The recombinant fungal host cell of any of Claims 24-37, wherein the host cell has had one or more native genes deleted from its genome.

39. The recombinant fungal host cell of Claim 38, wherein the deletion of said one or more native gene results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced production of by products, wherein comparison is made with respect to the corresponding host cell without the deletion(s).

40. The recombinant fungal host cell of any of Claims 24-39, wherein the host cell is altered to overexpress one or more polynucleotides.

41. The recombinant fungal host cell of Claim 40, wherein overexpression results in one or more phenotypes selected from increased transport of xylose into the host cell, increased xylulokinase activity, increased xylitol dehydrogenase activity, increased xylose isomerase activity, increased xylose reductase activity, increased flux through the pentose phosphate pathway, decreased sensitivity to catabolite repression, increased tolerance to ethanol, increased tolerance to acetate, increased tolerance to increased osmolarity, increased tolerance to low pH, and reduced product of by products, wherein comparison is made to the corresponding unaltered host cell.

42. The recombinant host cell of any of Claims 24-41, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

43. The recombinant host cell of any of Claims 24-42, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

44. The recombinant fungal host cell of any of Claims 24-43, wherein said nucleic acid construct comprising at least one polynucleotide sequence comprising at least one sequence selected from SEQ ID NOS:7, 9, 11, 13, 15, 17, 19, 21, and/or 23.

45. The recombinant fungal host cell of any of Claims 24-44, wherein said host cell is a yeast cell.

46. The recombinant fungal host cell of Claim 45, wherein said host cell is *Saccharomyces cerevisiae*.

47. A recombinant nucleic acid construct comprising at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase.

48. A recombinant nucleic acid construct comprising at least one polynucleotide sequence encoding at least one xylose isomerase, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase, and at least one polynucleotide sequence encoding at least one xylulokinase.

49. The recombinant nucleic acid construct of Claim 47 and/or 48, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic or prokaryotic enzymes.

50. The recombinant nucleic acid construct of any of Claims 47-49, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are eukaryotic enzymes.

51. The recombinant nucleic acid construct of any of Claims 47-50, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are fungal enzymes.

52. The recombinant nucleic acid construct of Claim 51, wherein said xylose isomerase, xylitol dehydrogenase, and xylulokinase are yeast enzymes

53. The recombinant nucleic acid construct of any of Claims 47-52, further comprising at least one genetic element that facilitates stable integration into a fungal host genome.

54. The recombinant nucleic acid construct of Claim 53, wherein said genetic element facilitates integration into a fungal host genome by homologous recombination.

55. The recombinant nucleic acid construct of Claim 53 and/or 54, wherein said genetic element comprises a prokaryotic or eukaryotic origin of replication and/or a centromeric plasmid maintenance sequence.

56. The recombinant nucleic acid construct of Claim 55, wherein the fungal origin of replication is a yeast origin of replication.

57. The recombinant nucleic acid construct of any of Claims 47-56, wherein said at least one polynucleotide sequence is operatively linked to a promoter sequence that is functional in a fungal cell.

58. The recombinant nucleic acid construct of any of Claims 47-57, wherein said at least one polynucleotide sequence encoding at least one xylose isomerase is operatively linked to a promoter sequence, at least one polynucleotide sequence encoding at least one xylitol dehydrogenase is operatively linked to a promoter sequence, and at least one polynucleotide sequence encoding at least one xylulokinase is operatively linked to a promoter sequence, wherein the promoter sequences are functional in a fungal host cell.

59. The recombinant nucleic acid construct of Claim 57 and/or 58, wherein said promoter sequence is a fungal promoter sequence.

60. The recombinant nucleic acid construct of Claim 59, wherein said fungal promoter sequence is a yeast promoter sequence.

61. The recombinant nucleic acid construct of any of Claims 47-60, wherein said polynucleotide sequence is operatively linked to at least one transcription termination sequence that is functional in a fungal cell.

62. The recombinant nucleic acid construct of any of Claims 47-61, wherein said polynucleotide sequence contains codons optimized for expression in a yeast cell.

63. The recombinant nucleic acid construct of any of Claims 47-62, wherein said construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

64. The recombinant nucleic acid construct of any of Claims 47-63, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; a polypeptide comprising an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

65. The recombinant nucleic acid construct of any of Claims 47-64, wherein said at least one polynucleotide sequence encodes at least one xylose isomerase, at least one xylitol dehydrogenase, and at least one xylulokinase, wherein the polynucleotide comprises at least one sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and 23; SEQ ID NO:3, and SEQ ID NO:5.

66. The recombinant nucleic acid construct of any of Claims 47-65, further comprising a polynucleotide sequence encoding at least one xylose reductase.

67. A recombinant nucleic acid construct comprising at least one polynucleotide sequence encoding at least one xylose isomerase.

68 The recombinant nucleic acid construct of Claim 67, wherein said xylose isomerase is a eukaryotic or prokaryotic enzyme.

69. The recombinant nucleic acid construct of Claim 67 and/or 68, wherein said xylose isomerase is a eukaryotic enzyme.

70. The recombinant nucleic acid construct of any of Claims 67-69, wherein said xylose isomerase is a fungal enzyme.

71. The recombinant nucleic acid construct of any of Claims 67-70, further comprising at least one genetic element that facilitates stable integration into a fungal host genome.

72. The recombinant nucleic acid construct of Claim 71, wherein said genetic element facilitates integration into a fungal host genome by homologous recombination.

73. The recombinant nucleic acid construct of Claim 71 and/or 72, wherein said genetic element comprises a fungal origin of replication.

74. The recombinant nucleic acid construct of Claim 73, wherein the fungal origin of replication is a yeast origin of replication.

75. The recombinant nucleic acid construct of any of Claims 67-74, wherein said at least one polynucleotide sequence encoding at least one xylose isomerase is operatively linked to a promoter sequence that is functional in a fungal host cell.

76. The recombinant nucleic acid construct of Claim 75, wherein said promoter sequence is a fungal promoter sequence.

77. The recombinant nucleic acid construct of Claim 76, wherein said fungal promoter sequence is a yeast promoter sequence.

78. The recombinant nucleic acid construct of any of Claims 67-77, wherein said at least one polynucleotide sequence is operatively linked to at least one transcription termination sequence that is functional in a fungal cell.

79. The recombinant nucleic acid construct of any of Claims 67-78, wherein said polynucleotide sequence contains codons optimized for expression in a yeast cell.

80. The recombinant nucleic acid construct of any of Claims 67-79, wherein said construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

81. The recombinant nucleic acid construct of any of Claims 67-80, wherein said nucleic acid construct comprises at least one polynucleotide sequence encoding at least one xylose isomerase, wherein the polynucleotide is selected from: (a) a polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24; and (b) a polynucleotide that hybridizes under stringent hybridization conditions to the complement of a polynucleotide that encodes a polypeptide having the amino acid sequence of SEQ ID NO:8, 10, 12, 14, 16, 18, 20, 22, and/or 24.

82. The recombinant nucleic acid construct of any of Claims 67-81, wherein said at least one polynucleotide sequence encodes at least one xylose isomerase, wherein the polynucleotide comprises at least one sequence selected from SEQ ID NOS:7, 9, 11, 13, 15, 17, 19, 21, and 23.

83. An isolated polypeptide sequence comprising a xylose isomerase polypeptide, xylitol dehydrogenase polypeptide, and xylulokinase polypeptide.

84. The isolated polypeptide sequence of Claim 83, wherein said xylose isomerase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least

about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; the xylitol dehydrogenase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and said xylulokinase polypeptide comprises an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6.

85. The isolated polypeptide sequence of Claim 83 and/or 84, wherein said xylose isomerase polypeptide comprises an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; said xylitol dehydrogenase polypeptide comprises an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and said xylulokinase polypeptide comprises an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6.

86. The isolated polypeptide sequence of any of Claims 83-85, wherein said xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and said xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5.

87. The isolated polypeptide sequence of any of Claims 83-86, wherein said xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; said xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and said xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5.

88. The isolated polypeptide sequence of any of Claims 83-87, wherein said xylose isomerase polypeptide is encoded by a polynucleotide sequence selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; said xylitol dehydrogenase polypeptide is encoded by SEQ ID NO:3; and said xylulokinase is encoded by SEQ ID NO:5.

89. An isolated polynucleotide sequence comprising a xylose isomerase polynucleotide, xylitol dehydrogenase polypeptide, and xylulokinase polypeptide.

90. The isolated polynucleotide sequence of Claim 89, wherein said xylose isomerase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:1, 7, 9, 12, 3, 15, 17, 19, 21, and/or 23; the xylitol dehydrogenase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:3; and said xylulokinase polynucleotide sequence has at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about

86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:5.

91. The isolated polynucleotide sequence of Claim 89 and/or 90, wherein said xylose isomerase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; said xylitol dehydrogenase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:3; and said xylulokinase polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence having 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 at least 100% identity to SEQ ID NO:5.

92. The isolated polynucleotide sequence of any of Claims 89-91, wherein said xylose isomerase polynucleotide sequence is selected from SEQ ID NOS:1, 7, 9, 11, 13, 15, 17, 19, 21, and/or 23; said xylitol dehydrogenase polynucleotide sequence is SEQ ID NO:3; and said xylulokinase polynucleotide sequence is SEQ ID NO:5.

93. The isolated polynucleotide sequence of any of Claims 89-92, wherein said xylose isomerase polynucleotide sequence encodes an amino acid sequence having at least about 70% at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least

about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; said xylitol dehydrogenase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:4; and said xylulokinase polynucleotide encodes an amino acid sequence having at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to SEQ ID NO:6.

94. The isolated polynucleotide sequence of any of Claims 89-93, wherein said xylose isomerase polynucleotide sequence encodes an amino acid sequence having at least 70%, 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 at least 100% identity to SEQ ID NO:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; said xylitol dehydrogenase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:4; and said xylulokinase polynucleotide encodes an amino acid sequence having 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 at least 100% identity to SEQ ID NO:6.

95. The isolated polynucleotide sequence of any of Claims 89-94, wherein said xylose isomerase polynucleotide sequence encodes an amino acid sequence selected from SEQ ID NOS:2, 8, 10, 12, 14, 16, 18, 20, 22, and/or 24; said xylitol dehydrogenase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:4; and said xylulokinase polynucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:6.

96. A method for producing a fermentation product, comprising:

(a) providing the recombinant fungal host cell of any of Claims 1 to 46;

(b) providing a fermentation medium; and

(c) contacting said fermentation medium with said recombinant fungal host cell under conditions suitable for generating said fermentation product.

97. The method of Claim 96, further comprising (d) recovering said fermentation product.

98. The method of Claim 96 and/or 97, wherein said fermenting step is carried out under conditions selected from anaerobic, microaerobic or aerobic conditions.

99. The method of any of Claims 96-98, wherein said fermentation product is selected from an alcohol, a fatty alcohol, a fatty acid, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, succinic acid, an amino acid, 1,3-propanediol, ethylene, glycerol, and a β-lactam.

100. The method of Claim 99, wherein said fermentation product is an alcohol selected from ethanol and butanol.

101. The method of Claim 100, wherein said fermentation product is ethanol.

102. The method of any of Claims 96-101, wherein said fermentation medium comprises product from a saccharification process.

103. The method of any of Claims 96-102, wherein said fermentation medium comprises hemicellulosic feedstock.

104. A method of producing at least one end product from at least one cellulosic substrate, comprising: a) providing at least one cellulosic substrate and at least one enzyme composition comprising at least one cellulase; b) contacting the cellulosic substrate with the enzyme composition under conditions whereby fermentable sugars are produced from the cellulosic substrate in a saccharification reaction; and c) contacting the fermentable sugars with a microorganism under fermentation conditions such that at least one end product is produced.

105. The method of Claim 104, wherein said method comprises simultaneous saccharification and fermentation reactions (SSF).

106. The method of Claim 104, wherein saccharification of the cellulosic substrate and said fermentation in separate reactions (SHF).

107. The method of Claim 104 and/or 105, wherein said enzyme composition is produced simultaneously with said saccharification reaction and said fermentation.

108. The method of any of Claims 104-107, further comprising at least one adjunct composition in said saccharification reaction.

109. The method of Claim 108, wherein said adjunct composition is selected from at least one divalent metal cation, copper, gallic acid, and/or at least one surfactant.

110. The method of any of Claims 96 to109, wherein said method is conducted at about pH 5.0.

111. The method of any of Claims 96 to 109, wherein said method is conducted at about pH 6.0.

112. The method of any of Claims 96 to 111, further comprising recovering at least one end product.

113. The method of any of Claims 96 to 112, wherein said end product comprises at least one fermentation end product.

114. The method of Claim 113, wherein said fermentation end product is selected from alcohols, fatty acids, lactic acid, acetic acid, 3-hydroxypropionic acid, acrylic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propanediol, ethylene, glycerol, fatty alcohols, butadiene, and beta-lactams.

115. The method of Claim 113 and/or 114, wherein said fermentation end product is at least one alcohol selected from ethanol and butanol.

116. The method of Claim 114 and/or 115, wherein said alcohol is ethanol.

117. The method of any of Claims 96 to 116, wherein the microorganism is a yeast.

118. The method of Claim 117, wherein the yeast is *Saccharomyces*.

119. The method of any of Claims 96 to 118, further comprising recovering at least one fermentation end product.

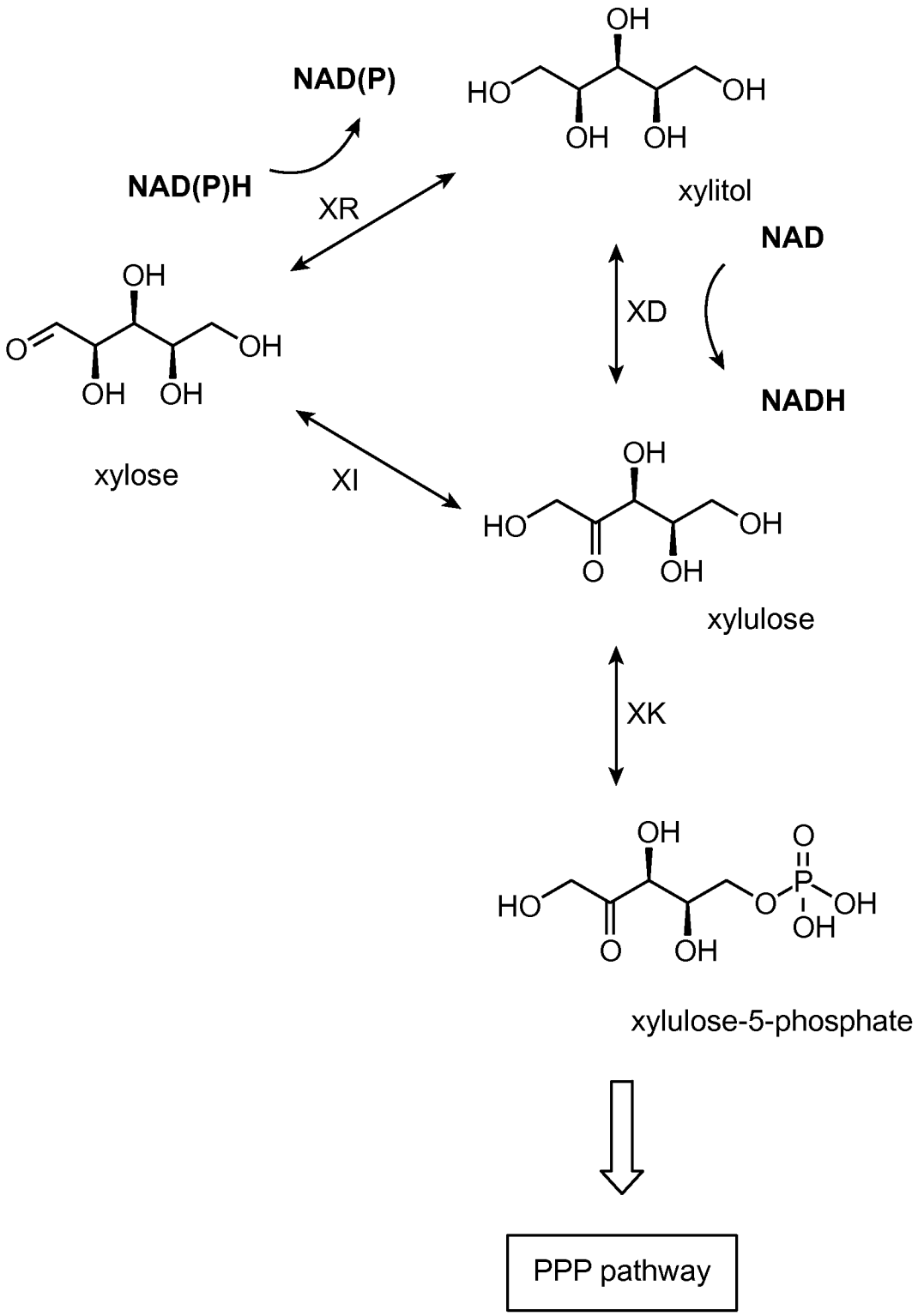
NAD(P)
NAD(P)H
XR
xylitol
NAD
XD
NADH
xylose
XI
xylulose
XK
xylulose-5-phosphate
PPP pathway

FIG. 1

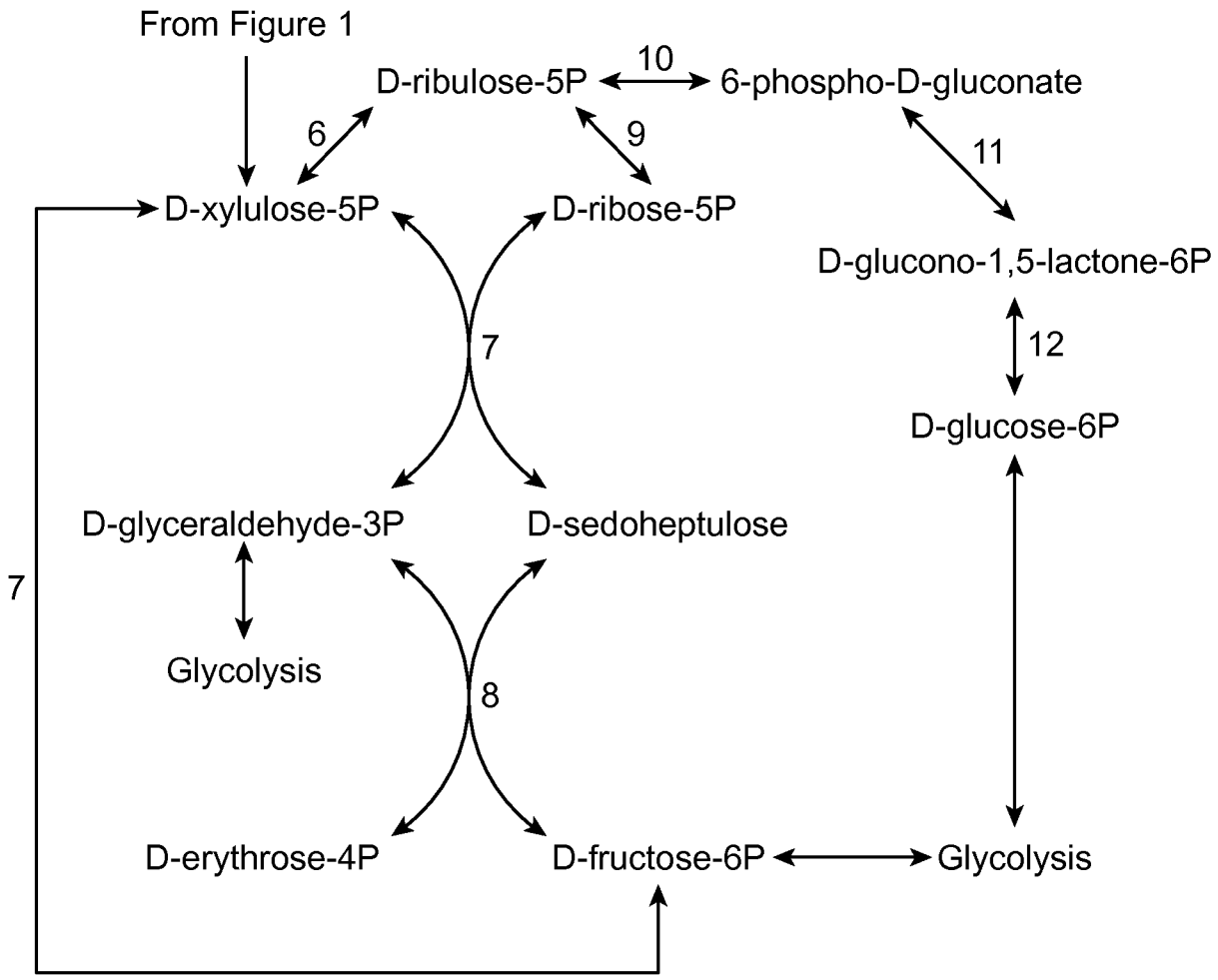
From Figure 1
D-ribulose-5P
10
6-phospho-D-gluconate
6
9
11
D-xylulose-5P
D-ribose-5P
D-glucono-1,5-lactone-6P
7
12
D-glucose-6P
D-glyceraldehyde-3P
D-sedoheptulose
7
Glycolysis
8
D-erythrose-4P
D-fructose-6P
Glycolysis

FIG. 2A

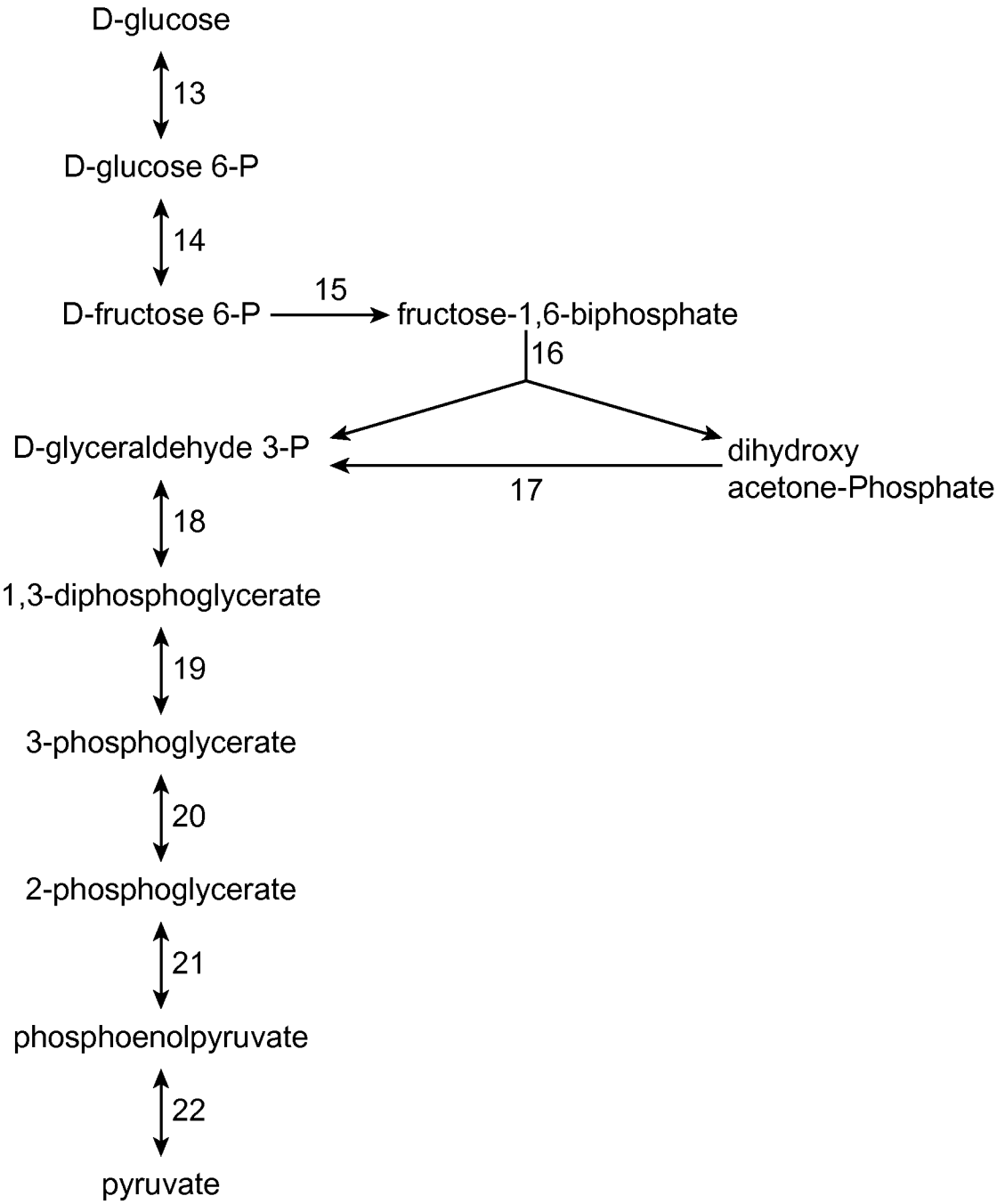
D-glucose
13
D-glucose 6-P
14
D-fructose 6-P
15
fructose-1,6-biphosphate
16
D-glyceraldehyde 3-P
dihydroxy
acetone-Phosphate
17
18
1,3-diphosphoglycerate
19
3-phosphoglycerate
20
2-phosphoglycerate
21
phosphoenolpyruvate
22
pyruvate

FIG. 2B

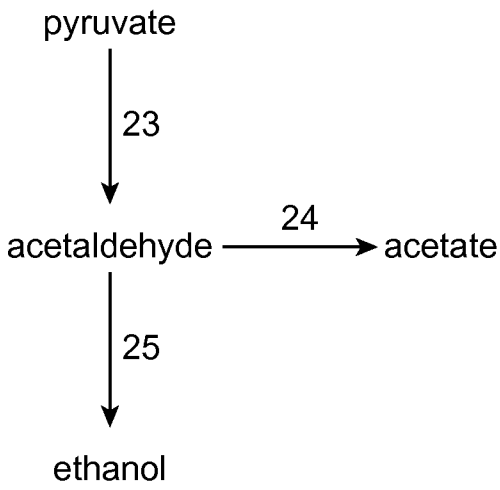

FIG. 2C

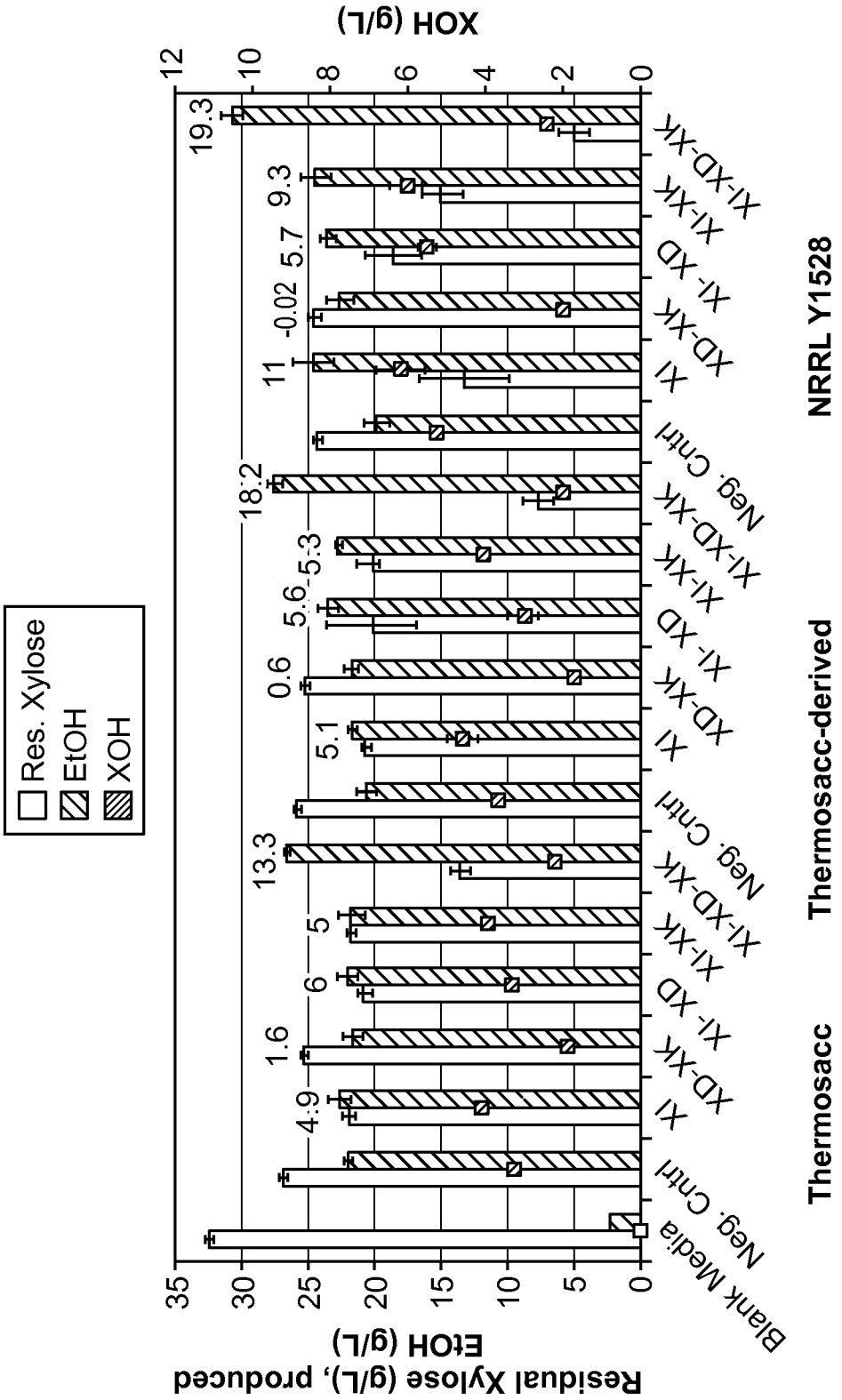
Residual Xylose (g/L), produced EtOH (g/L)
XOH (g/L)
Res. Xylose
EtOH
XOH
Blank Media
Neg. Cntrl
Xl
XD-XK
Xl-XD
Xl-XK
Xl-XD-XK
Neg. Cntrl
Xl
XD-XK
Xl-XD
Xl-XK
Xl-XD-XK
Neg. Cntrl
Xl
XD-XK
Xl-XD
Xl-XK
Xl-XD-XK
Thermosacc
Thermosacc-derived haploid
NRRL Y1528
4.9
1.6
6
5
13.3
5.1
0.6
5.6
5.3
18.2
11
-0.02
5.7
9.3
19.3

FIG. 3

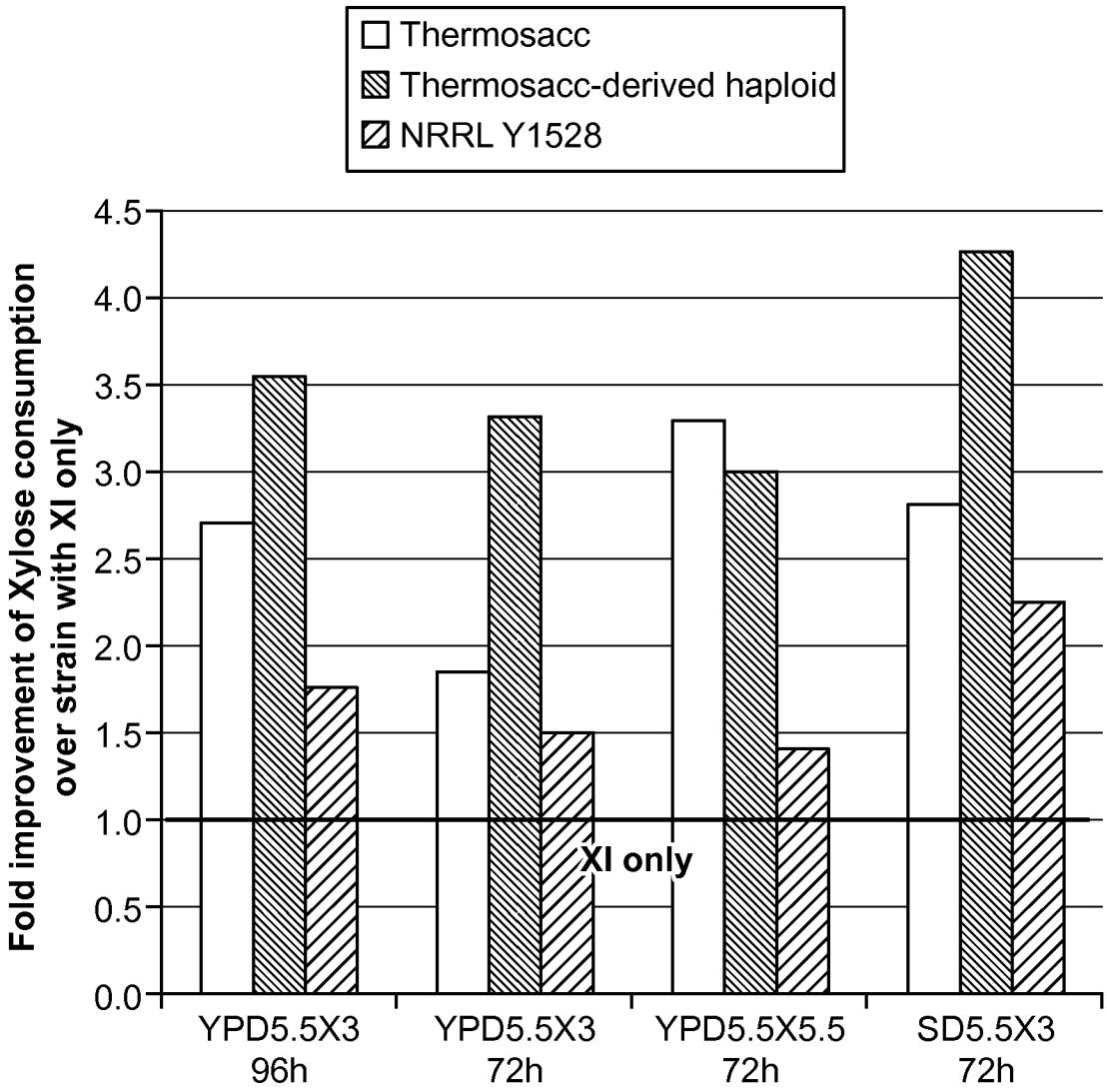
Thermosacc
Thermosacc-derived haploid
NRRL Y1528
Fold improvement of Xylose consumption over strain with XI only
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
XI only
YPD5.5X3 96h
YPD5.5X3 72h
YPD5.5X5.5 72h
SD5.5X3 72h

FIG. 4

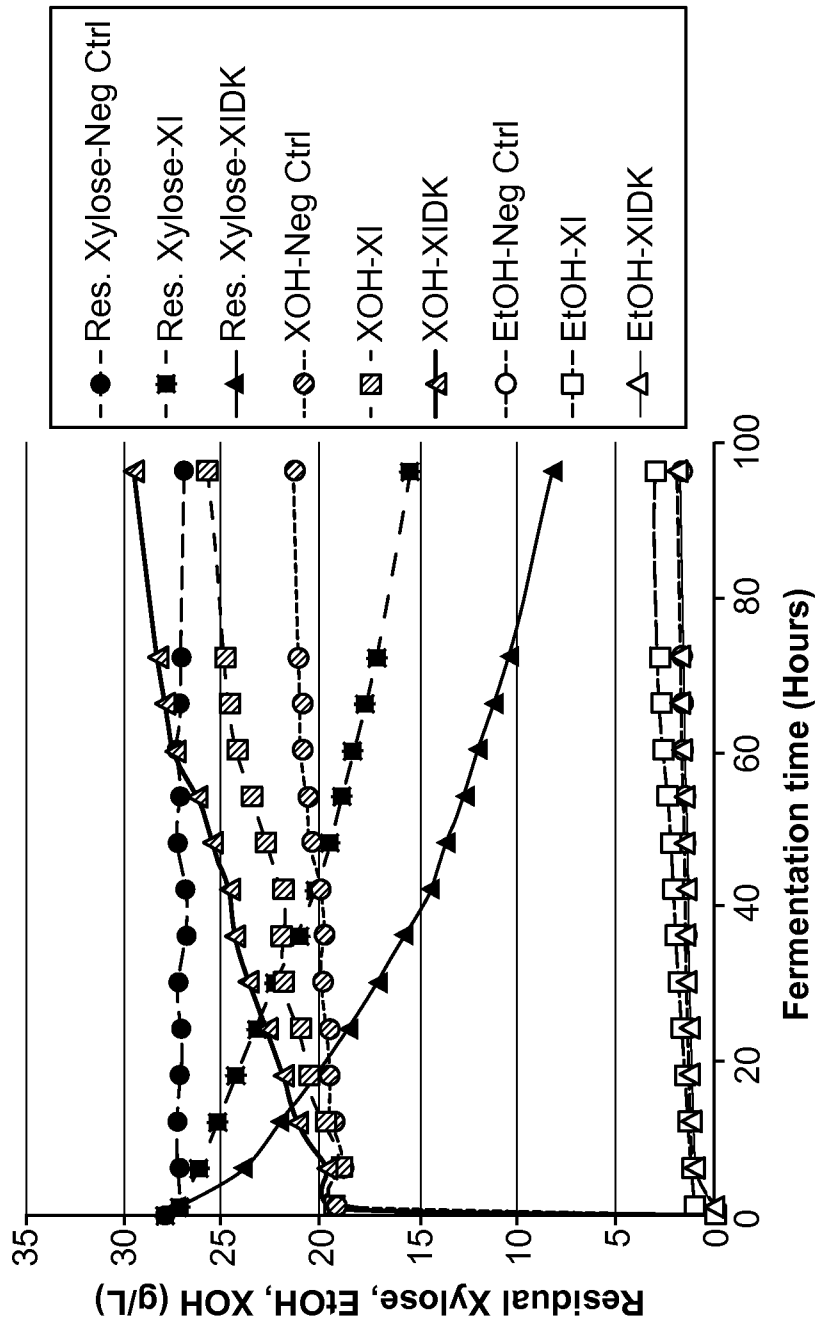
Res. Xylose-Neg Ctrl
Res. Xylose-XI
Res. Xylose-XIDK
XOH-Neg Ctrl
XOH-XI
XOH-XIDK
EtOH-Neg Ctrl
EtOH-XI
EtOH-XIDK
Residual Xylose, EtOH, XOH (g/L)
Fermentation time (Hours)
0
5
10
15
20
25
30
35
0
20
40
60
80
100

FIG. 5

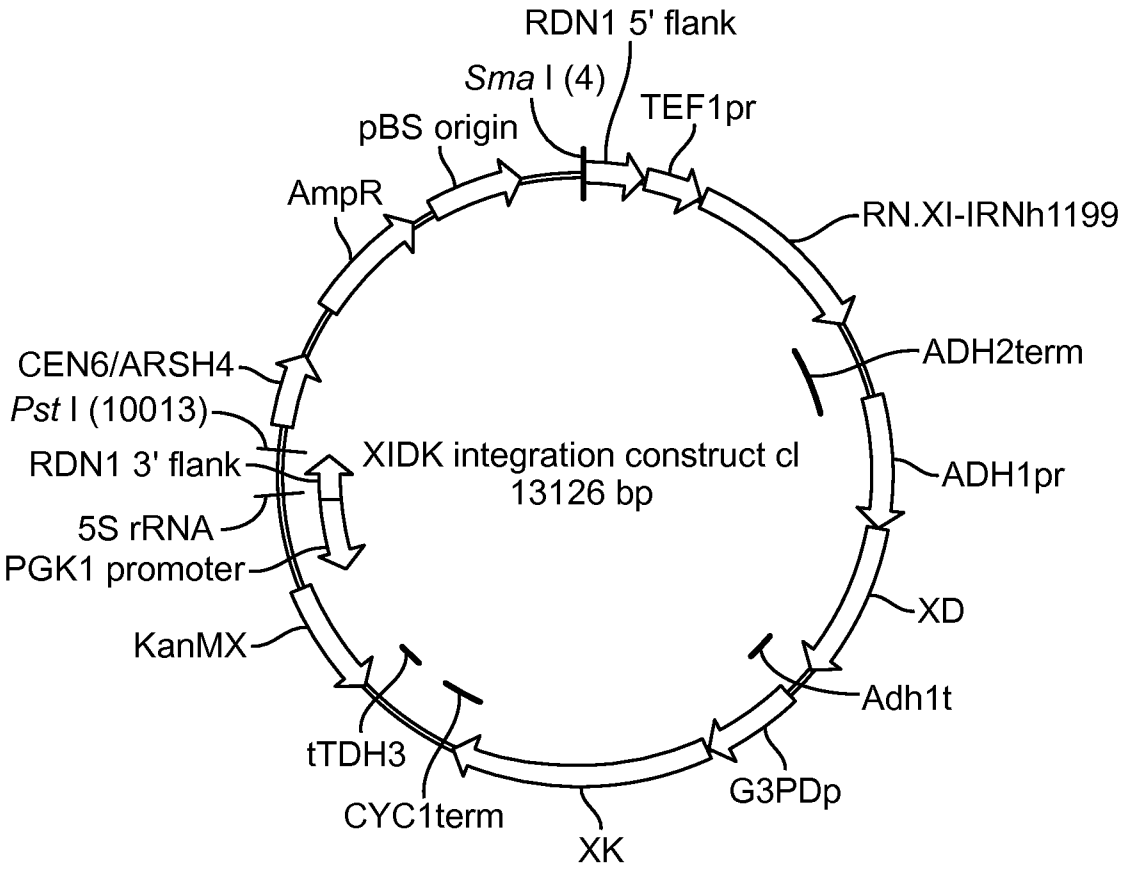
RDN1 5' flank
Sma I (4)
TEF1pr
pBS origin
AmpR
RN.XI-IRNh1199
ADH2term
CEN6/ARSH4
Pst I (10013)
RDN1 3' flank
XIDK integration construct cl
13126 bp
ADH1pr
5S rRNA
PGK1 promoter
XD
KanMX
Adh1t
tTDH3
G3PDp
CYC1term
XK

FIG. 6

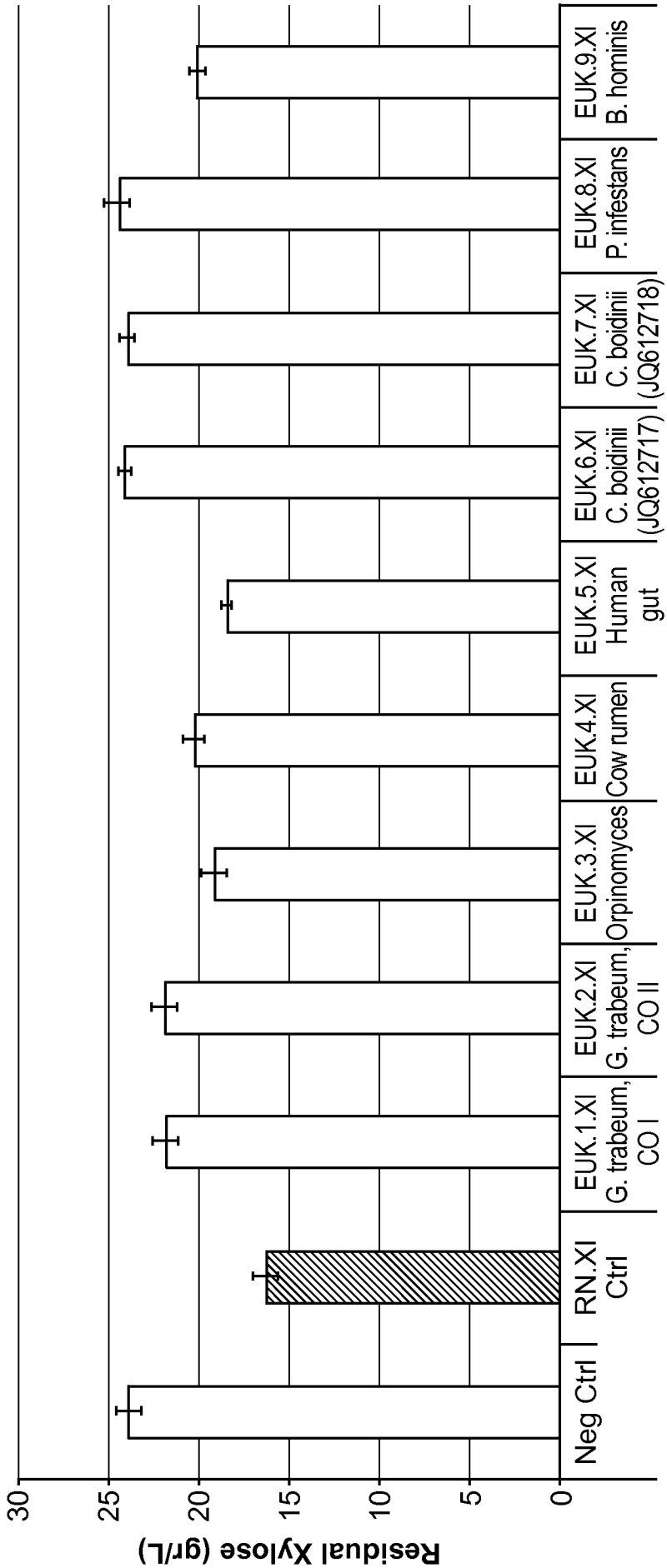
Residual Xylose (gr/L)
0
5
10
15
20
25
30
Neg Ctrl
RN.XI Ctrl
EUK.1.XI G. trabeum, CO I
EUK.2.XI G. trabeum, CO II
EUK.3.XI Orpinomyces
EUK.4.XI Cow rumen
EUK.5.XI Human gut
EUK.6.XI C. boidinii (JQ612717)
EUK.7.XI C. boidinii (JQ612718)
EUK.8.XI P. infestans
EUK.9.XI B. hominis

FIG. 7

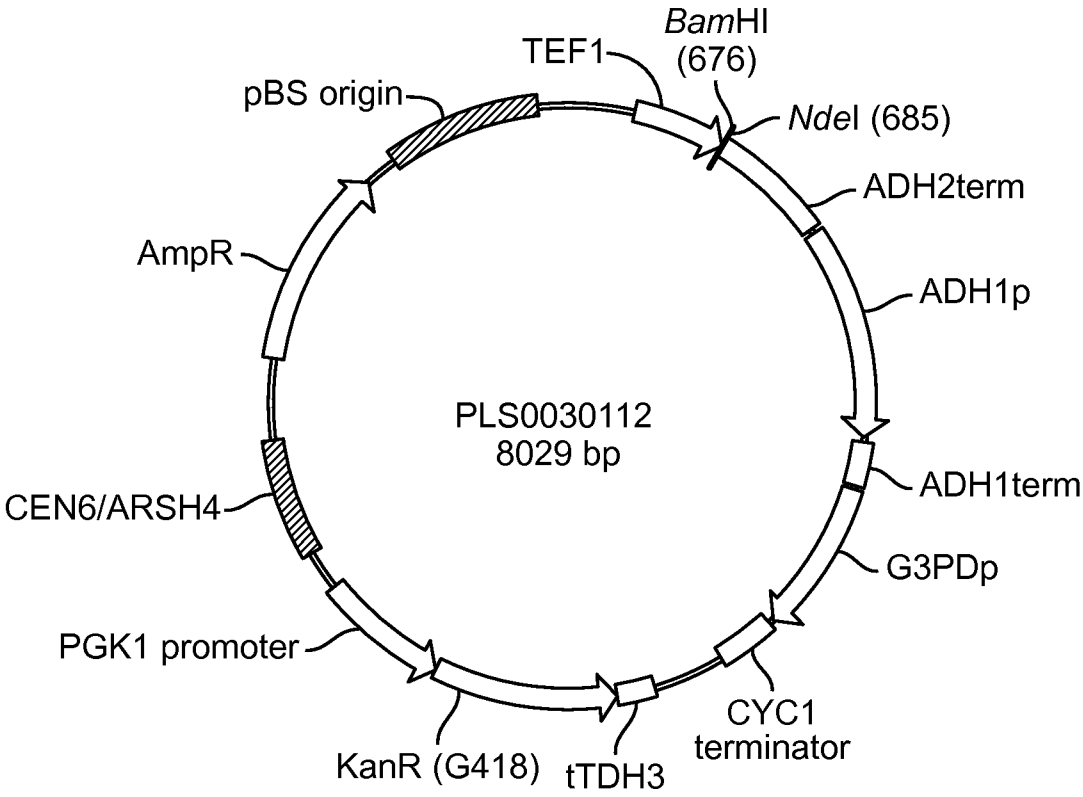
BamHI
(676)
TEF1
pBS origin
NdeI (685)
ADH2term
AmpR
ADH1p
PLS0030112
8029 bp
ADH1term
CEN6/ARSH4
G3PDp
PGK1 promoter
CYC1
terminator
KanR (G418)
tTDH3

FIG. 8

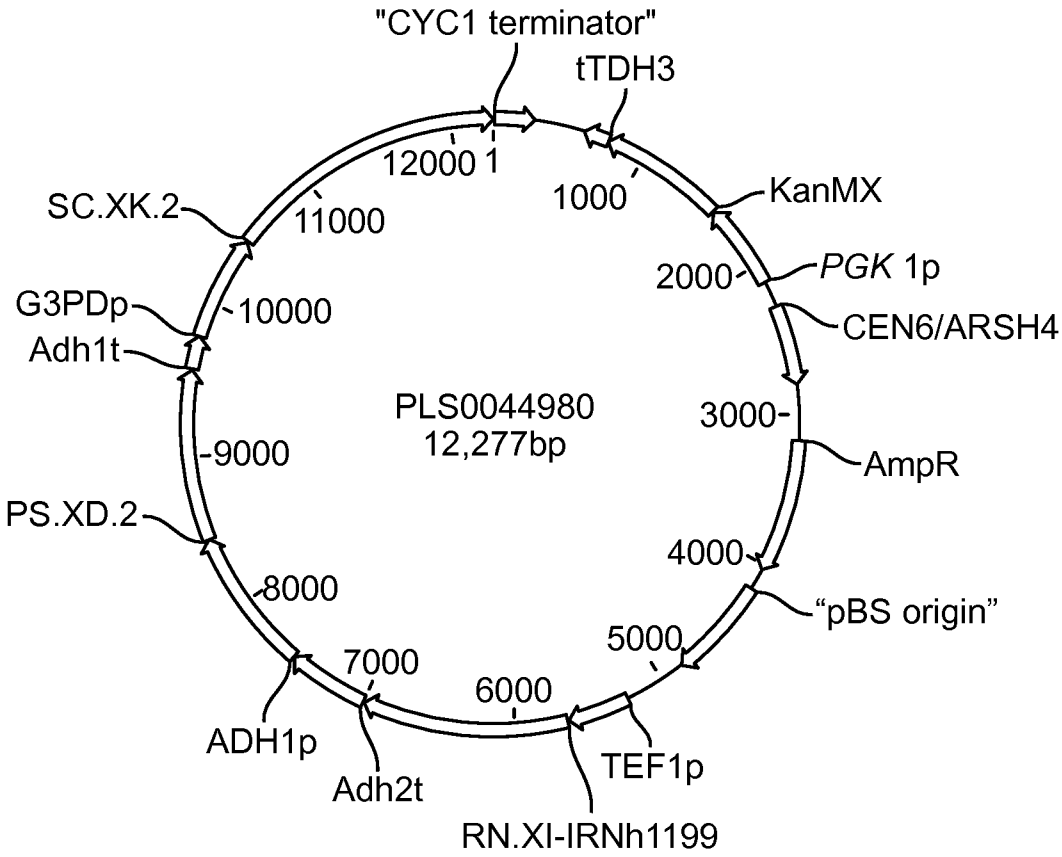
"CYC1 terminator"
tTDH3
12000 1
1000
KanMX
SC.XK.2
11000
2000
PGK 1p
G3PDp
10000
CEN6/ARSH4
Adh1t
PLS0044980
12,277bp
3000
AmpR
9000
PS.XD.2
4000
8000
"pBS origin"
5000
7000
6000
ADH1p
TEF1p
Adh2t
RN.XI-IRNh1199

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/070009

A. CLASSIFICATION OF SUBJECT MATTER
(see extra sheet)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C12N 15/11, 15/04, 9/24, 1/15, 1/18, C12P 7/06, C12N 9/92, C12P 19/24, C12R 1/645, 1/85, 1/865

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PAJ, Espacenet, PatSearch (RUPTO internal), USPTO DB, Patentscope, RUPTO, EAPATIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/070549 A (TERRANOL A/S et al.) 24.06.2010, pages 18, 20, 23, 26, 34, 36, claims	1- 6, 9, 10, 24-27, 47-50, 67-70, 83, 89, 96-99, 104-107
Y		7, 8
X	DATABASE UniProtKB/TrEMBL, A9KN98_CLOPH, 05.02.2008	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, B7SLY1_9FUNG, 10.02.2009	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, G1BER6_9ZZZ, 19.10.2011	83-86, 89-91

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 February 2014 (04.02.2014)	10 April 2014 (10.04.2014)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer E. Smirnova Telephone No. (495)531-65-15

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/070009

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProtKB/TrEMBL, D9ZEF8_9ZZZZ, 05.10.2010	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, I1VX39_CANBO, 11.07.2012	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, I1VX40_CANBO, 11.07.2012	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, D0NA42_PHYIT, 15.12.2009	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, D8MBL6_BLAHO, 05.10.2010	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, XKS1_YEAST, 01.10.1996	83-86, 89-91
X	DATABASE UniProtKB/TrEMBL, E5G64_PICSP, 08.02.2011	83-86, 89-91
Y	EP 479426 A1 (TONEN CORPORATION) 08.04.1992, abstract	7, 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2013/070009

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-23, 28-46, 51-66, 71-82, 87-88, 92-95, 100-103, 108-119
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

C12N 15/11 *(2006.01)*
C12N 15/04 *(2006.01)*
C12N 9/24 *(2006.01)*
C12N 1/15 *(2006.01)*
C12N 1/18 *(2006.01)*
C12P 7/06 *(2006.01)*
C12N 9/92 *(2006.01)*
C12P 19/24 *(2006.01)*
C12R 1/645 (2006.01)
C12R 1/85 (2006.01)
C12R 1/865 (2006.01)