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(54) **METABOLIC ENGINEERING OF ARABINOSE-FERMENTING YEAST CELLS**

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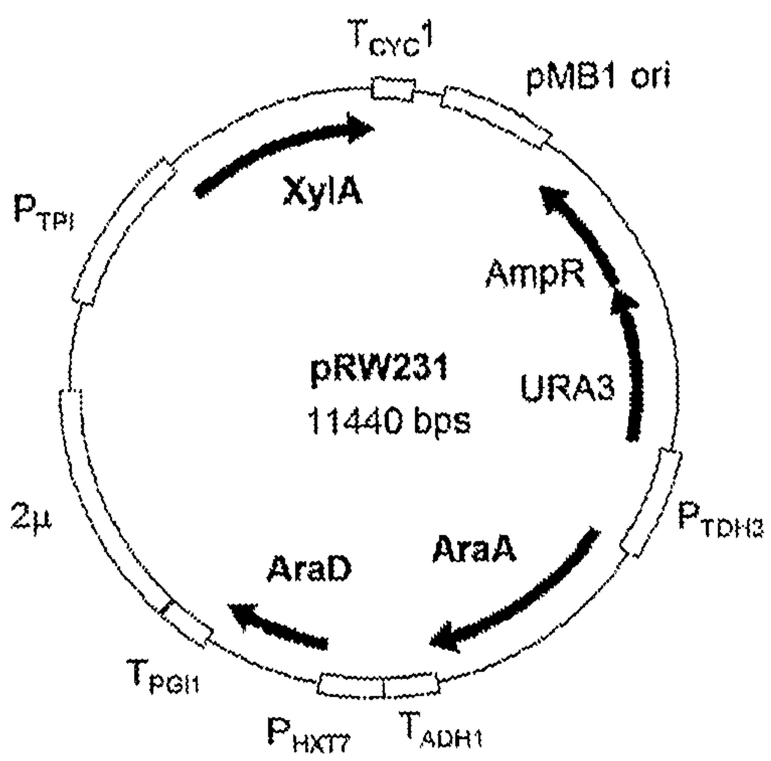
(57) **ABSTRACT**

The invention relates to an eukaryotic cell expressing nucleotide sequences encoding the ara A, ara B and ara D enzymes whereby the expression of these nucleotide sequences confers on the cell the ability to use L-arabinose and/or convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product such as ethanol. Optionally, the eukaryotic cell is also able to convert xylose into ethanol.



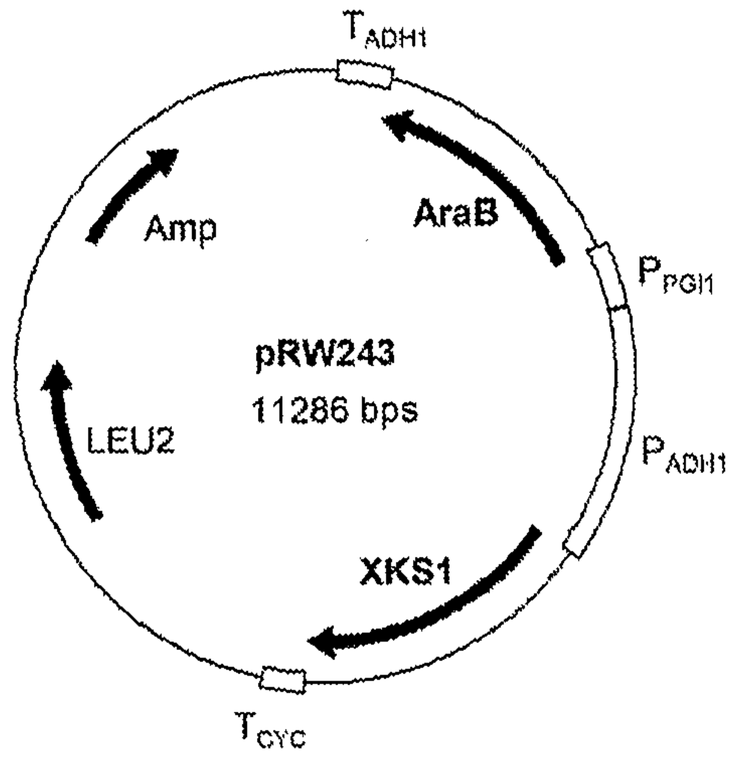


Fig 1

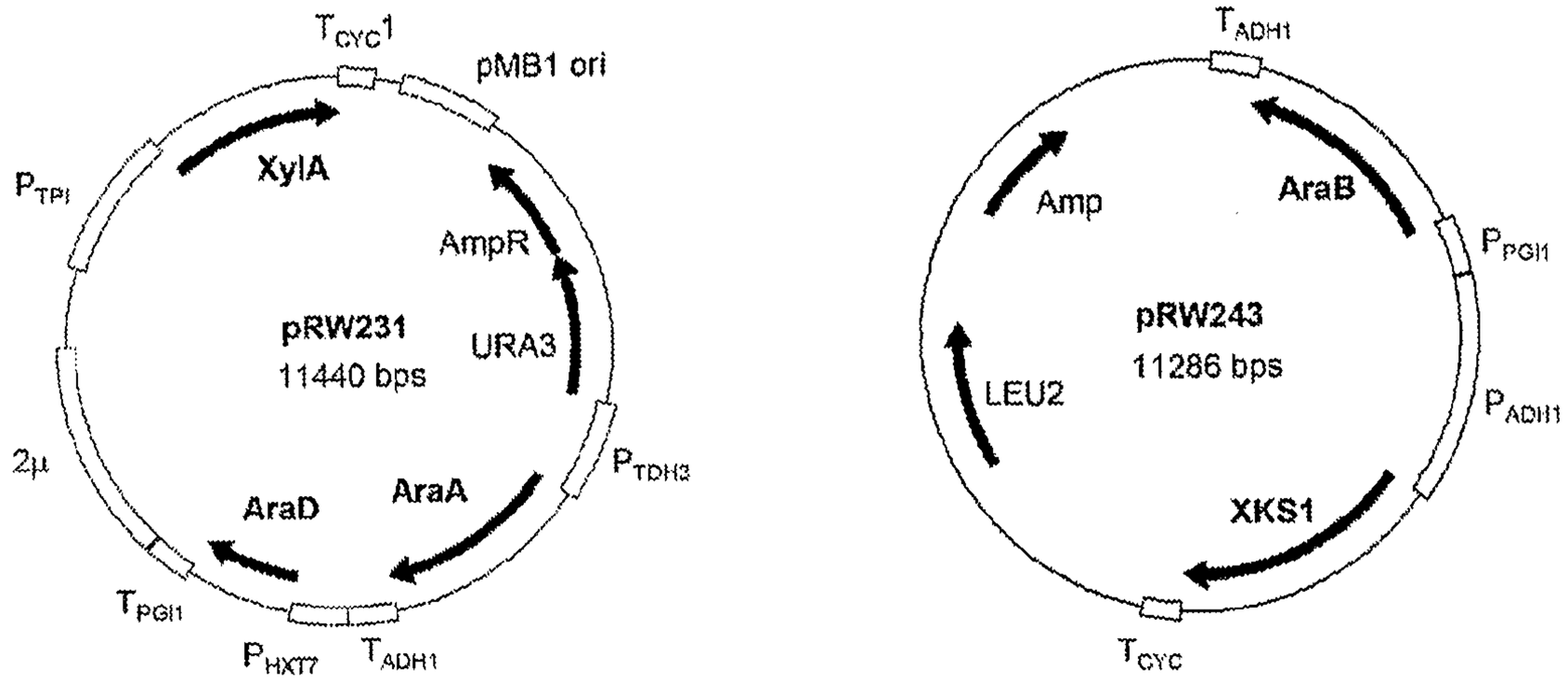


Fig 2

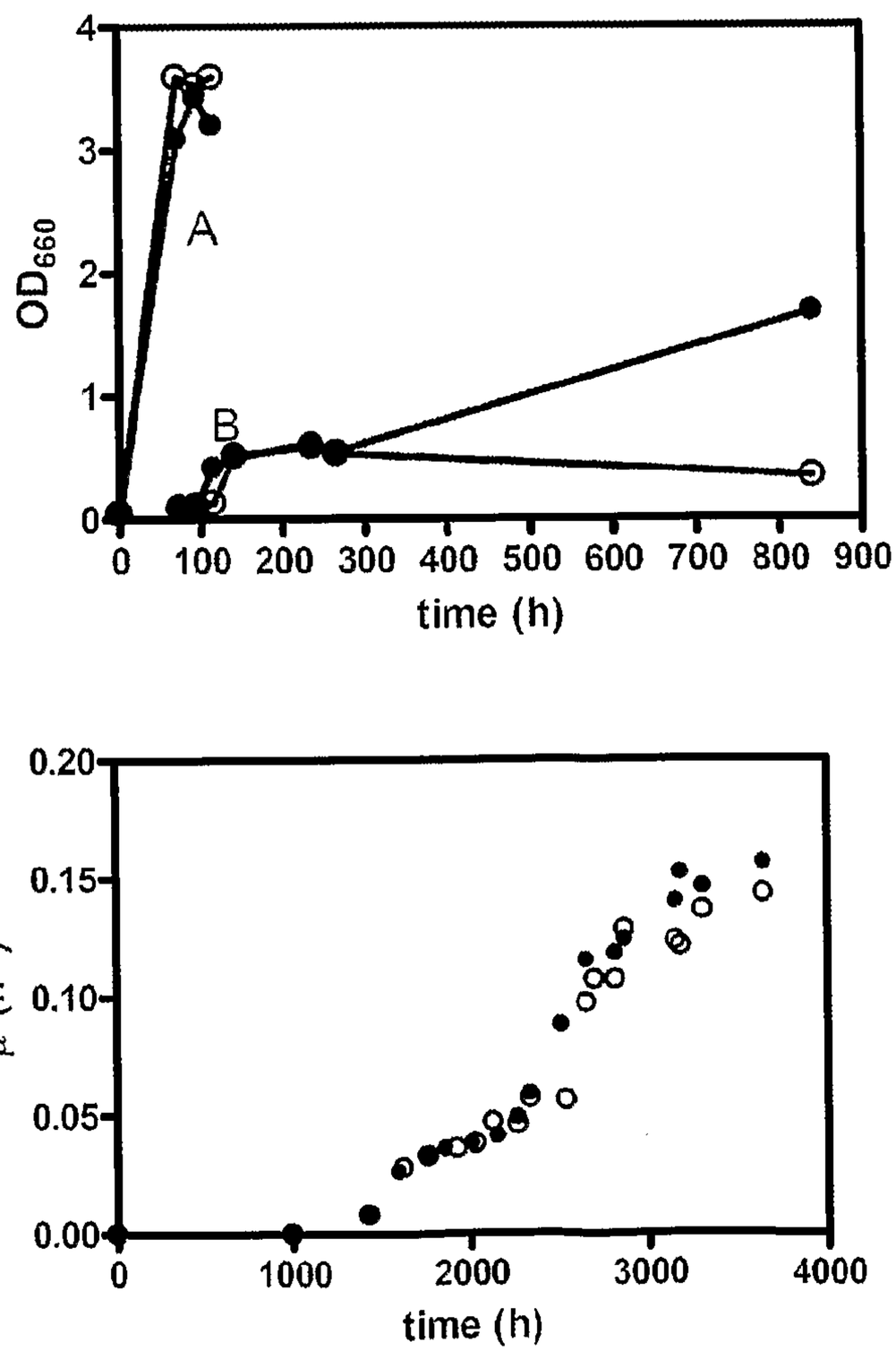


Fig 3

Fig 4

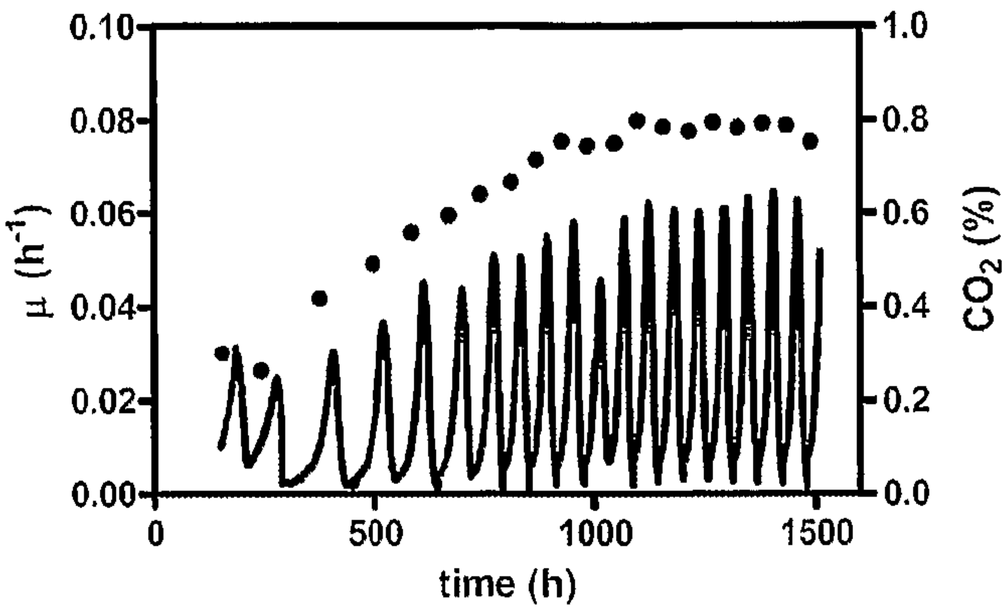


Fig 5a

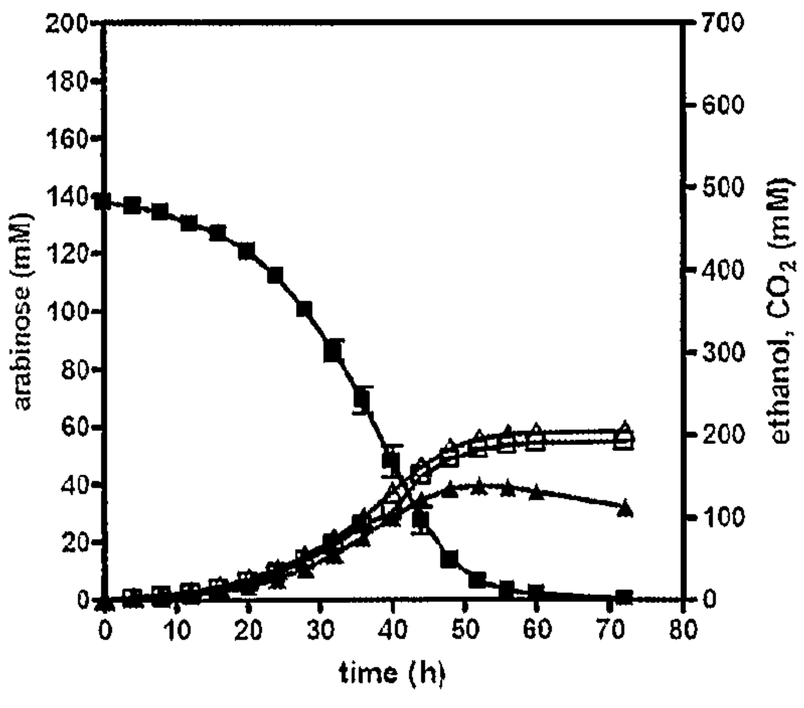


Fig 5b

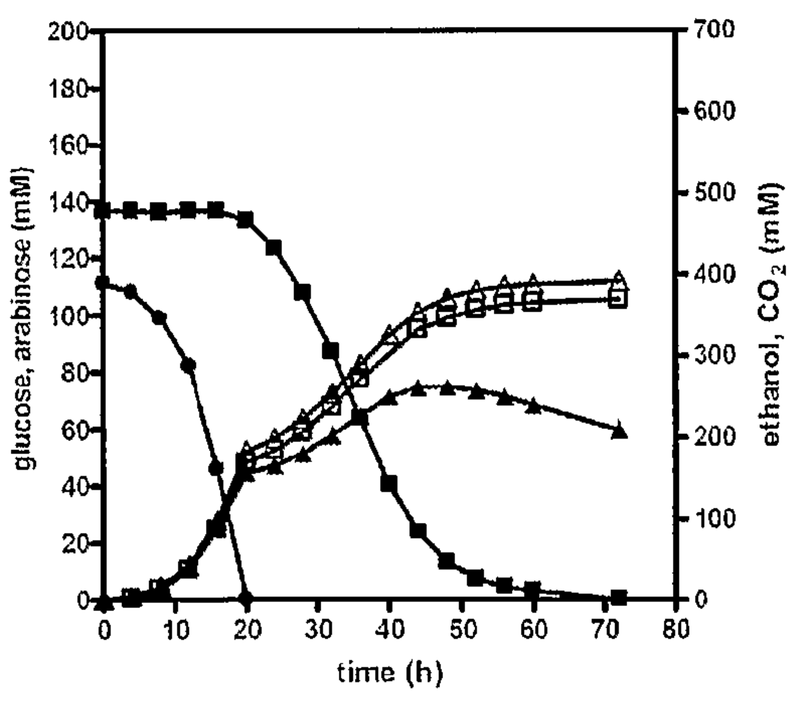


Fig 5c

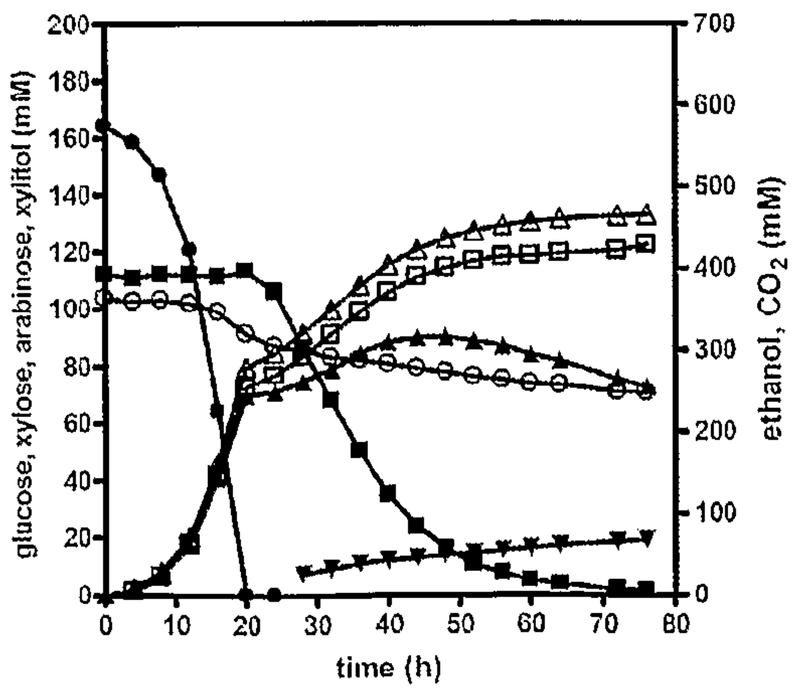


Fig 5d

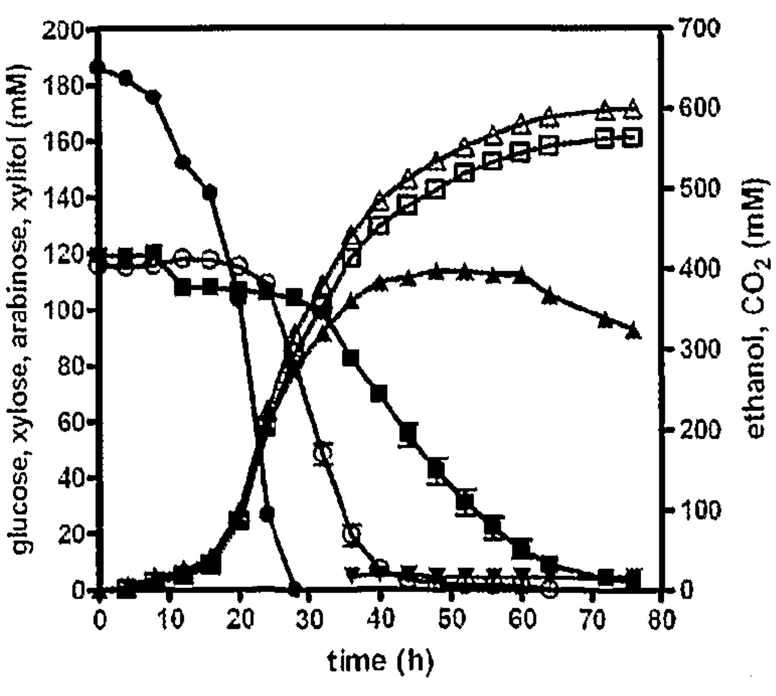
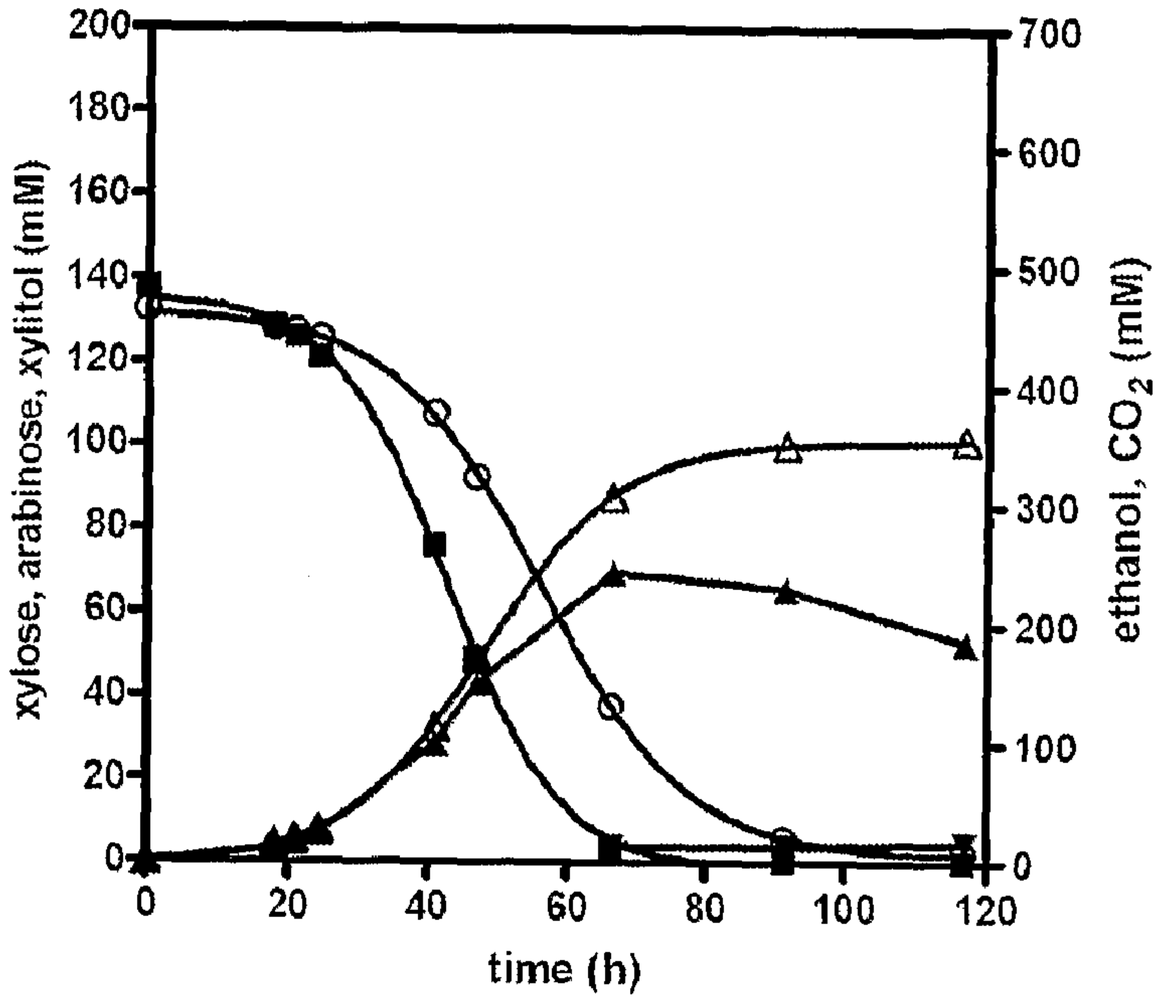


Fig 6


xylose, arabinose, xylitol (mM)
ethanol, CO_2 (mM)
time (h)
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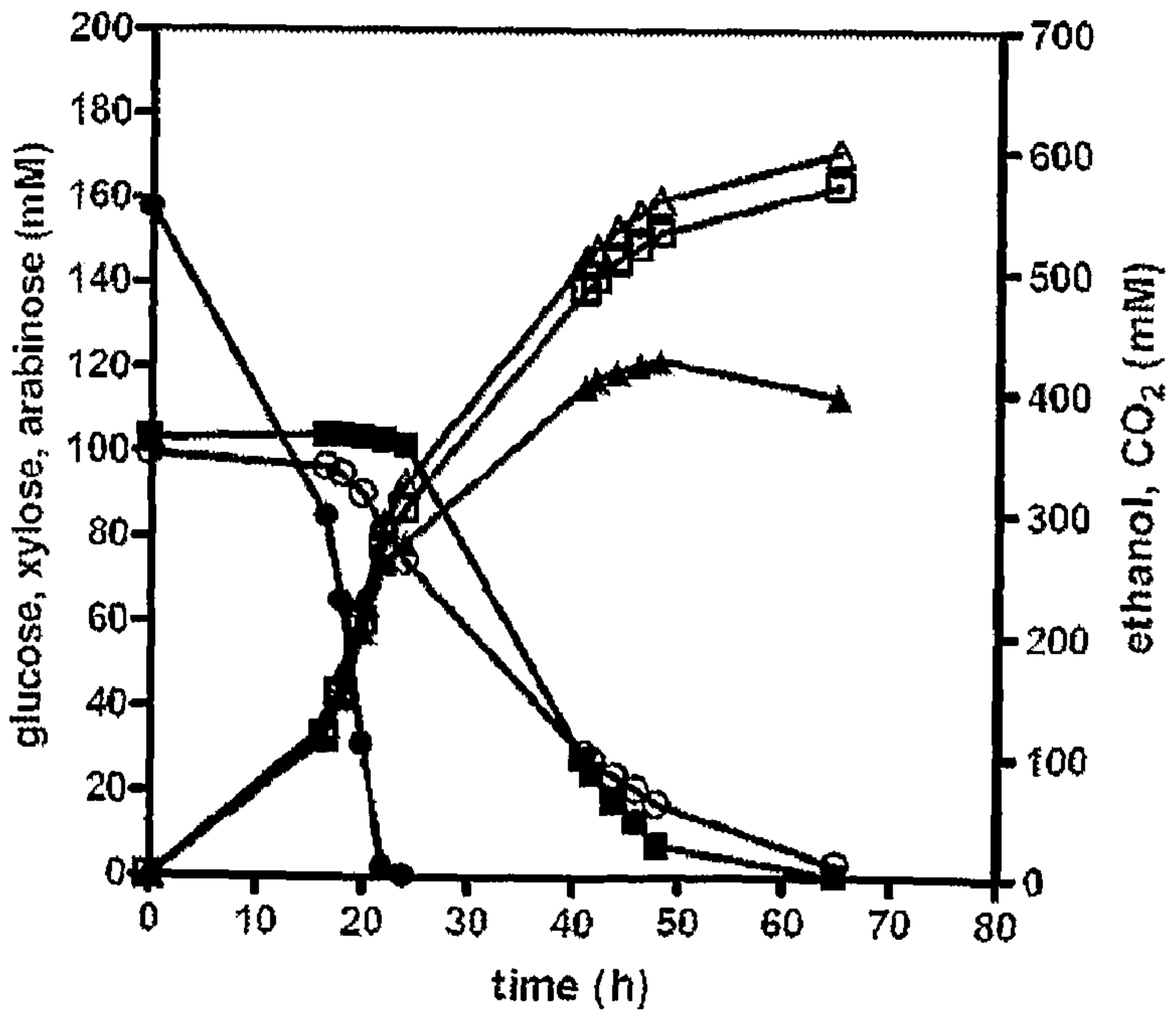
20
40
60
80
100
120
140
160
180
200
300
400
500
600
700

Fig 7


glucose, xylose, arabinose (mM)
ethanol, CO_2 (mM)
time (h)
0
10
20
30
40
50
60
70
80
100
120
140
160
180
200
300
400
500
600
700

METABOLIC ENGINEERING OF ARABINOSE-FERMENTING YEAST CELLS

FIELD OF THE INVENTION

[0001] The invention relates to an eukaryotic cell having the ability to use L-arabinose and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product and to a process for producing a fermentation product wherein this cell is used.

BACKGROUND OF THE INVENTION

[0002] Fuel ethanol is acknowledged as a valuable alternative to fossil fuels. Economically viable ethanol production from the hemicellulose fraction of plant biomass requires the simultaneous fermentative conversion of both pentoses and hexoses at comparable rates and with high yields. Yeasts, in particular *Saccharomyces* spp., are the most appropriate candidates for this process since they can grow and ferment fast on hexoses, both aerobically and anaerobically. Furthermore they are much more resistant to the toxic environment of lignocellulose hydrolysates than (genetically modified) bacteria.

[0003] EP 1 499 708 describes a process for making *S. cerevisiae* strains able to produce ethanol from L-arabinose. These strains were modified by introducing the araA (L-arabinose isomerase) gene from *Bacillus subtilis*, the araB (L-ribulokinase) and araD (L-ribulose-5-P4-epimerase) genes from *Escherichia coli*. Furthermore, these strains were either carrying additional mutations in their genome or overexpressing a TAL1 (transaldolase) gene. However, these strains have several drawbacks. They ferment arabinose in oxygen limited conditions. In addition, they have a low ethanol production rate of 0.05 $g \cdot g^{-1} \cdot h^{-1}$ (Becker and Boles, 2003). Furthermore, these strains are not able to use L-arabinose under anaerobic conditions. Finally, these *S. cerevisiae* strains have a wild type background, therefore they can not be used to co-ferment several C5 sugars.

[0004] WO 03/062430 and WO 06/009434 disclose yeast strains able to convert xylose into ethanol. These yeast strains are able to directly isomerise xylose into xylulose.

[0005] Still, there is a need for alternative strains for producing ethanol, which perform better and are more robust and resistant to relatively harsh production conditions.

DESCRIPTION OF THE FIGURES

[0006] FIG. **1**. Plasmid maps of pRW231 and pRW243.

[0007] FIG. **2**. Growth pattern of shake flask cultivations of strain RWB219 (○) and IMS0001 (●) in synthetic medium containing 0.5% galactose (A) and 0.1% galactose +2% L-arabinose (B). Cultures were grown for 72 hours in synthetic medium with galactose (A) and then transferred to synthetic medium with galactose and arabinose (B). Growth was determined by measuring the OD_{660}.

[0008] FIG. **3**. Growth rate during serial transfers of *S. cerevisiae* IMS0001 in shake flask cultures containing synthetic medium with 2% (w/v) L-arabinose. Each datapoint represents the growth rate estimated from the OD_{660} measured during (exponential) growth. The closed and open circles represent duplicate serial transfer experiments.

[0009] FIG. **4**. Growth rate during an anaerobic SBR fermentation of *S. cerevisiae* IMS0001 in synthetic medium with 2% (w/v) L-arabinose. Each datapoint represents the growth rate estimated from the CO_2 profile (solid line) during exponential growth.

[0010] FIG. **5**. Sugar consumption and product formation during anaerobic batch fermentations of strain IMS0002. The fermentations were performed in 1 synthetic medium supplemented with: 20 $g\,l^{-1}$ arabinose (A); 20 $g\,l^{-1}$ glucose and 20 $g\,l^{-1}$ arabinose (B); 30 $g\,l^{-1}$ glucose, 15 $g\,l^{-1}$ xylose, and 15 $g\,l^{-1}$ arabinose (C); Sugar consumption and product formation during anaerobic batch fermentations with a mixture of strains IMS0002 and RWB218. The fermentations were performed in 1 liter of synthetic medium supplemented with 30 $g\,l^{-1}$ glucose, 15 $g\,l^{-1}$ xylose, and 15 $g\,l^{-1}$ arabinose (D). Symbols: glucose (●); xylose (○); arabinose (■); ethanol calculated from cumulative CO_2 production (□); ethanol measured by HPLC (▲); cumulative CO_2 production (Δ); xylitol (▼)

[0011] FIG. **6**. Sugar consumption and product formation during an anaerobic batch fermentation of strain IMS0002 cells selected for anaerobic growth on xylose. The fermentation was performed in 1 liter of synthetic medium supplemented with 20 $g\,l^{-1}$ xylose and 20 $g\,l^{-1}$ arabinose. Symbols: xylose (○); arabinose (■); ethanol measured by HPLC (▲); cumulative CO_2 production (Δ); xylitol (▼).

[0012] FIG. **7**. Sugar consumption and product formation during an anaerobic batch fermentation of strain IMS0003. The fermentation was performed in 1 liter of synthetic medium supplemented with: 30 $g\,l^{-1}$ glucose, 15 $g\,l^{-1}$ xylose, and 15 $g\,l^{-1}$ arabinose. Symbols: glucose (●); xylose (○); arabinose (■); ethanol calculated from cumulative CO_2 production (□); ethanol measured by HPLC (▲); cumulative CO_2 production (Δ);

DESCRIPTION OF THE INVENTION

Eukaryotic Cell

[0013] In a first aspect, the invention relates to a eukaryotic cell capable of expressing the following nucleotide sequences, whereby the expression of these nucleotide sequences confers on the cell the ability to use L-arabinose and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product such as ethanol:

[0014] (a) a nucleotide sequence encoding an arabinose isomerase (araA), wherein said nucleotide sequence is selected from the group consisting of:

[0015] (i) nucleotide sequences encoding an araA, said araA comprising an amino acid sequence that has at least 55% sequence identity with the amino acid sequence of SEQ ID NO:1.

[0016] (ii) nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:2.

[0017] (iii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

[0018] (iv) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code,

[0019] (b) a nucleotide sequence encoding a L-ribulokinase (araB), wherein said nucleotide sequence is selected from the group consisting of:

[0020] (i) nucleotide sequences encoding an araB, said araB comprising an amino acid sequence that has

at least 20% sequence identity with the amino acid sequence of SEQ ID NO:3.

[0021] (ii) nucleotide sequences comprising a nucleotide sequence that has at least 50% sequence identity with the nucleotide sequence of SEQ ID NO:4.

[0022] (iii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

[0023] (iv) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code,

[0024] (c) a nucleotide sequence encoding an L-ribulose-5-P-4-epimerase (araD), wherein said nucleotide sequence is selected from the group consisting of:

[0025] (i) nucleotide sequences encoding an araD, said araD comprising an amino acid sequence that has at least 60% sequence identity with the amino acid sequence of SEQ ID NO:5.

[0026] (ii) nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:6.

[0027] (iii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

[0028] (iv) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code.

[0029] A preferred embodiment relates to an eukaryotic cell capable of expressing the following nucleotide sequences, whereby the expression of these nucleotide sequences confers on the cell the ability to use L-arabinose and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product such as ethanol:

[0030] (a) a nucleotide sequence encoding an arabinose isomerase (araA), wherein said nucleotide sequence is selected from the group consisting of:

[0031] (i) nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:2,

[0032] (ii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i);

[0033] (iii) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (ii) due to the degeneracy of the genetic code,

[0034] (b) a nucleotide sequence encoding a L-ribulokinase (araB), wherein said nucleotide sequence is selected from the group consisting of:

[0035] (i) nucleotide sequences encoding an araB, said araB comprising an amino acid sequence that has at least 20% sequence identity with the amino acid sequence of SEQ ID NO:3.

[0036] (ii) nucleotide sequences comprising a nucleotide sequence that has at least 50% sequence identity with the nucleotide sequence of SEQ ID NO:4.

[0037] (iii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

[0038] (iv) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code,

[0039] (c) a nucleotide sequence encoding an L-ribulose-5-P-4-epimerase (araD), wherein said nucleotide sequence is selected from the group consisting of:

[0040] (i) nucleotide sequences encoding an araD, said araD comprising an amino acid sequence that has at least 60% sequence identity with the amino acid sequence of SEQ ID NO:5.

[0041] (ii) nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:6.

[0042] (iii) nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

[0043] (iv) nucleotide sequences the sequences of which differ from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code.

Sequence Identity and Similarity

[0044] Sequence identity is herein defined as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. Usually, sequence identities or similarities are compared over the whole length of the sequences compared. In the art, "identity" also means the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. "Similarity" between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. "Identity" and "similarity" can be readily calculated by various methods, known to those skilled in the art.

[0045] Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include e.g. the BestFit, BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Mol. Biol. 215:403-410 (1990), publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894). A most preferred algorithm used is EMBOSS (http://www.ebi.ac.uk/emboss/align). Preferred parameters for amino acid sequences comparison using EMBOSS are gap open 10.0, gap extend 0.5, Blosum 62 matrix. Preferred parameters for nucleic acid sequences comparison using EMBOSS are gap open 10.0, gap extend 0.5, DNA full matrix (DNA identity matrix).

[0046] Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called "conservative" amino acid substitutions, as will be clear to the skilled person. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conser-

vative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids are as follows: Ala to ser; Arg to lys; Asn to gln or his; Asp to glu; Cys to ser or ala; Gln to asn; Glu to asp; Gly to pro; His to asn or gln; Ile to leu or val; Leu to ile or val; Lys to arg; gin or glu; Met to leu or ile; Phe to met, leu or tyr; Ser to thr; Thr to ser; Trp to tyr; Tyr to trp or phe; and, Val to ile or leu.

Hybridising Nucleic Acid Sequences

[0047] Nucleotide sequences encoding the enzymes expressed in the cell of the invention may also be defined by their capability to hybridise with the nucleotide sequences of SEQ ID NO.'s 2, 4, 6, 8, 16, 18, 20, 22, 24, 26, 28, 30 respectively, under moderate, or preferably under stringent hybridisation conditions. Stringent hybridisation conditions are herein defined as conditions that allow a nucleic acid sequence of at least about 25, preferably about 50 nucleotides, 75 or 100 and most preferably of about 200 or more nucleotides, to hybridise at a temperature of about 65° C. in a solution comprising about 1 M salt, preferably 6×SSC or any other solution having a comparable ionic strength, and washing at 65° C. in a solution comprising about 0.1 M salt, or less, preferably 0.2×SSC or any other solution having a comparable ionic strength. Preferably, the hybridisation is performed overnight, i.e. at least for 10 hours and preferably washing is performed for at least one hour with at least two changes of the washing solution. These conditions will usually allow the specific hybridisation of sequences having about 90% or more sequence identity.

[0048] Moderate conditions are herein defined as conditions that allow a nucleic acid sequences of at least 50 nucleotides, preferably of about 200 or more nucleotides, to hybridise at a temperature of about 45° C. in a solution comprising about 1 M salt, preferably 6×SSC or any other solution having a comparable ionic strength, and washing at room temperature in a solution comprising about 1 M salt, preferably 6×SSC or any other solution having a comparable ionic strength. Preferably, the hybridisation is performed overnight, i.e. at least for 10 hours, and preferably washing is performed for at least one hour with at least two changes of the washing solution. These conditions will usually allow the specific hybridisation of sequences having up to 50% sequence identity. The person skilled in the art will be able to modify these hybridisation conditions in order to specifically identify sequences varying in identity between 50% and 90%.

AraA

[0049] A preferred nucleotide sequence encoding a arabinose isomerase (araA) expressed in the cell of the invention is selected from the group consisting of:

[0050] (a) nucleotide sequences encoding an araA polypeptide said araA comprising an amino acid sequence that has at least 55, 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99% sequence identity with the amino acid sequence of SEQ ID NO. 1;

[0051] (b) nucleotide sequences comprising a nucleotide sequence that has at least 60, 70, 80, 90, 95, 97, 98, or 99% sequence identity with the nucleotide sequence of SEQ ID NO. 2;

[0052] (c) nucleotide sequences the complementary strand of which hybridises to a nucleic acid molecule sequence of (a) or (b);

[0053] (d) nucleotide sequences the sequence of which differ from the sequence of a nucleic acid molecule of (c) due to the degeneracy of the genetic code.

The nucleotide sequence encoding an araA may encode either a prokaryotic or an eukaryotic araA, i.e. an araA with an amino acid sequence that is identical to that of an araA that naturally occurs in the prokaryotic or eukaryotic organism. The present inventors have found that the ability of a particular araA to confer to a eukaryotic host cell the ability to use arabinose and/or to convert arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product such as ethanol when co-expressed with araB and araD does not depend so much on whether the araA is of prokaryotic or eukaryotic origin. Rather this depends on the relatedness of the araA's amino acid sequence to that of the sequence SEQ ID NO. 1.

AraB

[0054] A preferred nucleotide sequence encoding a L-ribulokinase (AraB) expressed in the cell of the invention is selected from the group consisting of:

[0055] (a) nucleotide sequences encoding a polypeptide comprising an amino acid sequence that has at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99% sequence identity with the amino acid sequence of SEQ ID NO. 3;

[0056] (b) nucleotide sequences comprising a nucleotide sequence that has at least 50, 60, 70, 80, 90, 95, 97, 98, or 99% sequence identity with the nucleotide sequence of SEQ ID NO.4;

[0057] (c) nucleotide sequences the complementary strand of which hybridises to a nucleic acid molecule sequence of (a) or (b);

[0058] (d) nucleotide sequences the sequence of which differ from the sequence of a nucleic acid molecule of (c) due to the degeneracy of the genetic code.

The nucleotide sequence encoding an araB may encode either a prokaryotic or an eukaryotic araB, i.e. an araB with an amino acid sequence that is identical to that of a araB that naturally occurs in the prokaryotic or eukaryotic organism. The present inventors have found that the ability of a particular araB to confer to a eukaryotic host cell the ability to use arabinose and/or to convert arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product when co-expressed with araA and araD does not depend so much on whether the araB is of prokaryotic or eukaryotic origin. Rather this depends on the relatedness of the araB's amino acid sequence to that of the sequence SEQ ID NO. 3.

AraD

[0059] A preferred nucleotide sequence encoding a L-ribulose-5-P-4-epimerase (araD) expressed in the cell of the invention is selected from the group consisting of:

[0060] (e) nucleotide sequences encoding a polypeptide comprising an amino acid sequence that has at least 60,

65, 70, 75, 80, 85, 90, 95, 97, 98, or 99% sequence identity with the amino acid sequence of SEQ ID NO. 5;

[0061] (f) nucleotide sequences comprising a nucleotide sequence that has at least 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99% sequence identity with the nucleotide sequence of SEQ ID NO.6;

[0062] (g) nucleotide sequences the complementary strand of which hybridises to a nucleic acid molecule sequence of (a) or (b);

[0063] (h) nucleotide sequences the sequence of which differs from the sequence of a nucleic acid molecule of (c) due to the degeneracy of the genetic code.

The nucleotide sequence encoding an araD may encode either a prokaryotic or an eukaryotic araD, i.e. an araD with an amino acid sequence that is identical to that of a araD that naturally occurs in the prokaryotic or eukaryotic organism. The present inventors have found that the ability of a particular araD to confer to a eukaryotic host cell the ability to use arabinose and/or to convert arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product when co-expressed with araA and araB does not depend so much on whether the araD is of prokaryotic or eukaryotic origin. Rather this depends on the relatedness of the araD's amino acid sequence to that of the sequence SEQ ID NO. 5.

[0064] Surprisingly, the codon bias index indicated that expression of the *Lactobacillus plantarum* araA, araB and araD genes were more favorable for expression in yeast than the prokaryolic araA, araB and araD genes described in EP 1 499 708.

[0065] It is to be noted that *L. plantarum* is a Generally Regarded As Safe (GRAS) organism, which is recognized as safe by food registration authorities. Therefore, a preferred nucleotide sequence encodes an araA, araB or araD respectively having an amino acid sequence that is related to the sequences SEQ ID NO: 1, 3, or 5 respectively as defined above. A preferred nucleotide sequence encodes a fungal araA, araB or araD respectively (e.g. from a Basidiomycete), more preferably an araA, araB or araD respectively from an anaerobic fungus, e.g. an anaerobic fungus that belongs to the families Neocallimastix, Caecomyces, Pfromyces, Orpinomyces, or Ruminomyces. Alternatively, a preferred nucleotide sequence encodes a bacterial araA, araB or araD respectively, preferably from a Gram-positive bacterium, more preferably from the genus *Lactobacillus*, most preferably from *Lactobacillus plantarum* species. Preferably, one, two or three or the araA, araB and araD nucleotide sequences originate from a *Lactobacillus* genus, more preferably a *Lactobacillus plantarum* species. The bacterial araA expressed in the cell of the invention is not the *Bacillus subtilis* araA disclosed in EP 1 499 708 and given as SEQ ID NO:9. SEQ ID NO:10 represents the nucleotide acid sequence coding for SEQ ID NO:9. The bacterial araB and araD expressed in the cell of the invention are not the ones of *Escherichia coli* (*E. coli*) as disclosed in EP 1 499 708 and given as SEQ ID NO: 11 and SEQ ID NO:13. SEQ ID NO: 12 represents the nucleotide acid sequence coding for SEQ ID NO:11. SEQ ID NO:14 represents the nucleotide acid sequence coding for SEQ ID NO:13.

[0066] To increase the likelihood that the (bacterial) araA, araB and araD enzymes respectively are expressed in active form in a eukaryotic host cell of the invention such as yeast, the corresponding encoding nucleotide sequence may be adapted to optimise its codon usage to that of the chosen eukaryotic host cell. The adaptiveness of a nucleotide sequence encoding the araA, araB, and araD enzymes (or other enzymes of the invention, see below) to the codon usage of the chosen host cell may be expressed as codon adaptation index (CAI). The codon adaptation index is herein defined as a measurement of the relative adaptiveness of the codon usage of a gene towards the codon usage of highly expressed genes. The relative adaptiveness (w) of each codon is the ratio of the usage of each codon, to that of the most abundant codon for the same amino acid. The CAI index is defined as the geometric mean of these relative adaptiveness values. Non-synonymous codons and termination codons (dependent on genetic code) are excluded. CAI values range from 0 to 1, with higher values indicating a higher proportion of the most abundant codons (see Sharp and Li, 1987, Nucleic Acids Research 15: 1281-1295; also see: Jansen et al., 2003, Nucleic Acids Res. 31(8):2242-51). An adapted nucleotide sequence preferably has a CAI of at least 0.2, 0.3, 0.4, 0.5, 0.6 or 0.7.

[0067] In a preferred embodiment, expression of the nucleotide sequences encoding an ara A, an ara B and an ara D as defined earlier herein confers to the cell the ability to use L-arabinose and/or to convert it into L-ribulose, and/or xylulose 5-phosphate. Without wishing to be bound by any theory, L-arabinose is expected to be first converted into L-ribulose, which is subsequently converted into xylulose 5-phosphate which is the main molecule entering the pentose phosphate pathway. In the context of the invention, "using L-arabinose" preferably means that the optical density measured at 660 nm (OD_{660}) of transformed cells cultured under aerobic or anaerobic conditions in the presence of at least 0.5% L-arabinose during at least 20 days is increased from approximately 0.5 till 1.0 or more. More preferably, the OD_{660} is increased from 0.5 till 1.5 or more. More preferably, the cells are cultured in the presence of at least 1%, at least 1.5%, at least 2% L-arabinose. Most preferably, the cells are cultured in the presence of approximately 2% L-arabinose.

[0068] In the context of the invention, a cell is able "to convert L-arabinose into L-ribulose" when detectable amounts of L-ribulose are detected in cells cultured under aerobic or anaerobic conditions in the presence of L-arabinose (same preferred concentrations as in previous paragraph) during at least 20 days using a suitable assay. Preferably the assay is HPLC for L-ribulose.

[0069] In the context of the invention, a cell is able "to convert L-arabinose into xylulose 5-phosphate" when an increase of at least 2% of xylulose 5-phosphate is detected in cells cultured under aerobic or anaerobic conditions in the presence of L-arabinose (same preferred concentrations as in previous paragraph) during at least 20 days using a suitable assay. Preferably, an HPCL-based assay for xylulose 5-phosphate has been described in Zaldivar J., et al ((2002), Appl. Microbiol. Biotechnol., 59:436-442). This assay is briefly described in the experimental part. More preferably, the increase is of at least 5%, 10%, 15%, 20%, 25% or more.

[0070] In another preferred embodiment, expression of the nucleotide sequences encoding an ara A, ara B and ara D as defined earlier herein confers to the cell the ability to convert L-arabinose into a desired fermentation product when cultured under aerobic or anaerobic conditions in the presence of L-arabinose (same preferred concentrations as in previous paragraph) during at least one month till one year. More preferably, a cell is able to convert L-arabinose into a desired fermentation product when detectable amounts of a desired

fermentation product are detected using a suitable assay and when the cells are cultured under the conditions given in previous sentence. Even more preferably, the assay is HPLC. Even more preferably, the fermentation product is ethanol.

[0071] A cell for transformation with the nucleotide sequences encoding the araA, araB, and araD enzymes respectively as described above, preferably is a host cell capable of active or passive xylose transport into and xylose isomerisation within the cell. The cell preferably is capable of active glycolysis. The cell may further contain an endogenous pentose phosphate pathway and may contain endogenous xylulose kinase activity so that xylulose isomerised from xylose may be metabolised to pyruvate. The cell further preferably contains enzymes for conversion of pyruvate to a desired fermentation product such as ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic or a cephalosporin. The cell may be made capable of producing butanol by introduction of one or more genes of the butanol pathway as disclosed in WO2007/041269.

[0072] A preferred cell is naturally capable of alcoholic fermentation, preferably, anaerobic alcoholic fermentation. The host cell further preferably has a high tolerance to ethanol, a high tolerance to low pH (i.e. capable of growth at a pH lower than 5, 4, 3, or 2.5) and towards organic acids like lactic acid, acetic acid or formic acid and sugar degradation products such as furfural and hydroxy-methylfurfural, and a high tolerance to elevated temperatures. Any of these characteristics or activities of the host cell may be naturally present in the host cell or may be introduced or modified through genetic selection or by genetic modification. A suitable host cell is a eukaryotic microorganism like e.g. a fungus, however, most suitable as host cell are yeasts or filamentous fungi.

[0073] Yeasts are herein defined as eukaryotic microorganisms and include all species of the subdivision Eumycotina (Alexopoulos, C. J., 1962, In: Introductory Mycology, John Wiley & Sons, Inc., New York) that predominantly grow in unicellular form. Yeasts may either grow by budding of a unicellular thallus or may grow by fission of the organism. Preferred yeasts as host cells belong to one of the genera *Saccharomyces, Kluyveromyces, Candida, Pichia, Schizosaccharomyces, Hansenula, Kloeckera, Schwanniomyces*, or *Yarrowia*. Preferably the yeast is capable of anaerobic fermentation, more preferably anaerobic alcoholic fermentation.

[0074] Filamentous fungi are herein defined as eukaryotic microorganisms that include all filamentous forms of the subdivision Eumycotina. These fungi are characterized by a vegetative mycelium composed of chitin, cellulose, and other complex polysaccharides. The filamentous fungi of the present invention are morphologically, physiologically, and genetically distinct from yeasts. Vegetative growth by filamentous fungi is by hyphal elongation and carbon catabolism of most filamentous fungi is obligately aerobic. Preferred filamentous fungi as host cells belong to one of the genera *Aspergillus, Trichoderma, Humicola, Acremonium, Fusarium*, or *Penicillium*.

[0075] Over the years suggestions have been made for the introduction of various organisms for the production of bio-ethanol from crop sugars. In practice, however, all major bio-ethanol production processes have continued to use the yeasts of the genus *Saccharomyces* as ethanol producer. This is due to the many attractive features of *Saccharomyces* species for industrial processes, i.e., a high acid-, ethanol- and osmo-tolerance, capability of anaerobic growth, and of course its high alcoholic fermentative capacity. Preferred yeast species as host cells include *S. cerevisiae, S. bulderi, S. barnetti, S. exiguus, S. uvarum, S. diastaticus, K. lactis, K. marxianus, K. fragilis*.

[0076] In a preferred embodiment, the host cell of the invention is a host cell that has been transformed with a nucleic acid construct comprising the nucleotide sequence encoding the araA, araB, and araD enzymes as defined above. In one more preferred embodiment, the host cell is co-transformed with three nucleic acid constructs, each nucleic acid construct comprising the nucleotide sequence encoding araA, araB or araD. The nucleic acid construct comprising the araA, araB, and/or araD coding sequence is capable of expression of the araA, araB, and/or araD enzymes in the host cell. To this end the nucleic acid construct may be constructed as described in e.g. WO 03/0624430. The host cell may comprise a single but preferably comprises multiple copies of each nucleic acid construct. The nucleic acid construct may be maintained episomally and thus comprise a sequence for autonomous replication, such as an ARS sequence. Suitable episomal nucleic acid constructs may e.g. be based on the yeast 2μ or pKD1 (Fleer et al., 1991, Biotechnology 9:968-975) plasmids. Preferably, however, each nucleic acid construct is integrated in one or more copies into the genome of the host cell. Integration into the host cell's genome may occur at random by illegitimate recombination but preferably nucleic acid construct is integrated into the host cell's genome by homologous recombination as is well known in the art of fungal molecular genetics (see e.g. WO 90/14423, EP-A-0 481 008, EP-A-0 635 574 and U.S. Pat. No. 6,265,186). Accordingly, in a more preferred embodiment, the cell of the invention comprises a nucleic acid construct comprising the araA, araB, and/or araD coding sequence and is capable of expression of the araA, araB, and/or araD enzymes. In an even more preferred embodiment, the araA, araB, and/or araD coding sequences are each operably linked to a promoter that causes sufficient expression of the corresponding nucleotide sequences in a cell to confer to the cell the ability to use L-arabinose, and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate. Preferably the cell is a yeast cell. Accordingly, in a further aspect, the invention also encompasses a nucleic acid construct as earlier outlined herein. Preferably, a nucleic acid construct comprises a nucleic acid sequence encoding an araA, araB and/or araD. Nucleic acid sequences encoding an araA, araB, or araD have been all earlier defined herein. Even more preferably, the expression of the corresponding nucleotide sequences in a cell confer to the cell the ability to convert L-arabinose into a desired fermentation product as defined later herein. In an even more preferred embodiment, the fermentation product is ethanol. Even more preferably, the cell is a yeast cell.

[0077] As used herein, the term "operably linked" refers to a linkage of polynucleotide elements (or coding sequences or nucleic acid sequence) in a functional relationship. A nucleic acid sequence is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the nucleic acid sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

[0078] As used herein, the term "promoter" refers to a nucleic acid fragment that functions to control the transcription of one or more genes, located upstream with respect to the direction of transcription of the transcription initiation site of the gene, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation.

[0079] The promoter that could be used to achieve the expression of the nucleotide sequences coding for araA, araB and/or araD may be not native to the nucleotide sequence coding for the enzyme to be expressed, i.e. a promoter that is heterologous to the nucleotide sequence (coding sequence) to which it is operably linked. Although the promoter preferably is heterologous to the coding sequence to which it is operably linked, it is also preferred that the promoter is homologous, i.e. endogenous to the host cell. Preferably the heterologous promoter (to the nucleotide sequence) is capable of producing a higher steady state level of the transcript comprising the coding sequence (or is capable of producing more transcript molecules, i.e. mRNA molecules, per unit of time) than is the promoter that is native to the coding sequence, preferably under conditions where arabinose, or arabinose and glucose, or xylose and arabinose or xylose and arabinose and glucose are available as carbon sources, more preferably as major carbon sources (i.e. more than 50% of the available carbon source consists of arabinose, or arabinose and glucose, or xylose and arabinose or xylose and arabinose and glucose), most preferably as sole carbon sources. Suitable promoters in this context include both constitutive and inducible natural promoters as well as engineered promoters. A preferred promoter for use in the present invention will in addition be insensitive to catabolite (glucose) repression and/or will preferably not require arabinose and/or xylose for induction.

[0080] Promotors having these characteristics are widely available and known to the skilled person. Suitable examples of such promoters include e.g. promoters from glycolytic genes, such as the phosphofructokinase (PPK), triose phosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GPD, TDH3 or GAPDH), pyruvate kinase (PYK), phosphoglycerate kinase (PGK) promoters from yeasts or filamentous fungi; more details about such promoters from yeast may be found in (WO 93/03159). Other useful promoters are ribosomal protein encoding gene promoters, the lactase gene promoter (LAC4), alcohol dehydrogenase promoters (ADH1, ADH4, and the like), the enolase promoter (ENO), the glucose-6-phosphate isomerase promoter (PGI1, Hauf et al, 2000) or the hexose(glucose) transporter promoter (HXT7) or the glyceraldehyde-3-phosphate dehydrogenase (TDH3). The sequence of the PGI1 promoter is given in SEQ ID NO:51. The sequence of the HXT7 promoter is given in SEQ ID NO:52. The sequence of the TDH3 promoter is given in SEQ ID NO:49. Other promoters, both constitutive and inducible, and enhancers or upstream activating sequences will be known to those of skill in the art. The promoters used in the host cells of the invention may be modified, if desired, to affect their control characteristics. A preferred cell of the invention is a eukaryotic cell transformed with the araA, araB and araD genes of *L. plantarum*. More preferably, the eukaryotic cell is a yeast cell, even more preferably a *S. cerevisiae* strain transformed with the araA, araB and araD genes of *L. plantarum*. Most preferably, the cell is either CBS120327 or CBS120328 both deposited at the CBS Institute (The Netherlands) on Sep. 27, 2006.

[0081] The term "homologous" when used to indicate the relation between a given (recombinant) nucleic acid or polypeptide molecule and a given host organism or host cell, is understood to mean that in nature the nucleic acid or polypeptide molecule is produced by a host cell or organisms of the same species, preferably of the same variety or strain. If homologous to a host cell, a nucleic acid sequence encoding a polypeptide will typically be operably linked to another promoter sequence or, if applicable, another secretory signal sequence and/or terminator sequence than in its natural environment. When used to indicate the relatedness of two nucleic acid sequences the term "homologous" means that one single-stranded nucleic acid sequence may hybridize to a complementary single-stranded nucleic acid sequence. The degree of hybridization may depend on a number of factors including the amount of identity between the sequences and the hybridization conditions such as temperature and salt concentration as earlier presented. Preferably the region of identity is greater than about 5 bp, more preferably the region of identity is greater than 10 bp.

[0082] The term "heterologous" when used with respect to a nucleic acid (DNA or RNA) or protein refers to a nucleic acid or protein that does not occur naturally as part of the organism, cell, genome or DNA or RNA sequence in which it is present, or that is found in a cell or location or locations in the genome or DNA or RNA sequence that differ from that in which it is found in nature. Heterologous nucleic acids or proteins are not endogenous to the cell into which it is introduced, but has been obtained from another cell or synthetically or recombinantly produced. Generally, though not necessarily, such nucleic acids encode proteins that are not normally produced by the cell in which the DNA is transcribed or expressed. Similarly exogenous RNA encodes for proteins not normally expressed in the cell in which the exogenous RNA is present. Heterologous nucleic acids and proteins may also be referred to as foreign nucleic acids or proteins. Any nucleic acid or protein that one of skill in the art would recognize as heterologous or foreign to the cell in which it is expressed is herein encompassed by the term heterologous nucleic acid or protein. The term heterologous also applies to non-natural combinations of nucleic acid or amino acid sequences, i.e. combinations where at least two of the combined sequences are foreign with respect to each other.

Preferred Eukaryotic Cell Able to Use and/or Convert L-Arabinose and Xylose

[0083] In a more preferred embodiment, the cell of the invention that expresses araA, araB and araD is able to use L-arabinose and/or to convert it into L-ribulose, and/or xylulose 5-phosphate and/or a desired fermentation product as earlier defined herein and additionally exhibits the ability to use xylose and/or convert xylose into xylulose. The conversion of xylose into xylulose is preferably a one step isomerisation step (direct isomerisation of xylose into xylulose). This type of cell is therefore able to use both L-arabinose and xylose. "Using" xylose has preferably the same meaning as "using" L-arabinose as earlier defined herein.

[0084] Enzyme definitions are as used in WO 06/009434, for xylose isomerase (EC 5.3.1.5), xylulose kinase (EC 2.7.1.17), ribulose 5-phosphate epimerase (5.1.3.1), ribulose 5-phosphate isomerase (EC 5.3.1.6), transketolase (EC 2.2.1.1), transaldolase (EC 2.2.1.2), and aldose reductase" (EC 1.1.1.21).

[0085] In a preferred embodiment, the eukaryotic cell of the invention expressing araA, araB and araD as earlier defined herein has the ability of isomerising xylose to xylulose as e.g. described in WO 03/0624430 or in WO 06/009434. The ability of isomerising xylose to xylulose is conferred to the host cell by transformation of the host cell with a nucleic acid construct comprising a nucleotide sequence encoding a xylose isomerase. The transformed host cell's ability to isomerise xylose into xylulose is the direct isomerisation of xylose to xylulose. This is understood to mean that xylose isomerised into xylulose in a single reaction catalysed by a xylose isomerase, as opposed to the two step conversion of xylose into xylulose via a xylitol intermediate as catalysed by xylose reductase and xylitol dehydrogenase, respectively.

[0086] The nucleotide sequence encodes a xylose isomerase that is preferably expressed in active form in the transformed host cell of the invention. Thus, expression of the nucleotide sequence in the host cell produces a xylose isomerase with a specific activity of at least 10 U xylose isomerase activity per mg protein at 30° C., preferably at least 20, 25, 30, 50, 100, 200, 300 or 500 U per mg at 30° C. The specific activity of the xylose isomerase expressed in the transformed host cell is herein defined as the amount of xylose isomerase activity units per mg protein of cell free lysate of the host cell, e.g. a yeast cell free lysate. Determination of the xylose isomerase activity has already been described earlier herein.

[0087] Preferably, expression of the nucleotide sequence encoding the xylose isomerase in the host cell produces a xylose isomerase with a K_m for xylose that is less than 50, 40, 30 or 25 mM, more preferably, the K_m for xylose is about 20 mM or less.

[0088] A preferred nucleotide sequence encoding the xylose isomerase may be selected from the group consisting of:

[0089] (e) nucleotide sequences encoding a polypeptide comprising an amino acid sequence that has at least 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, or 99% sequence identity with the amino acid sequence of SEQ ID NO. 7 or SEQ ID NO:15;

[0090] (f) nucleotide sequences comprising a nucleotide sequence that has at least 40, 50, 60, 70, 80, 90, 95, 97, 98, or 99% sequence identity with the nucleotide sequence of SEQ ID NO. 8 or SEQ ID NO:16;

[0091] (g) nucleotide sequences the complementary strand of which hybridises to a nucleic acid molecule sequence of (a) or (b);

[0092] (h) nucleotide sequences the sequence of which differs from the sequence of a nucleic acid molecule of (c) due to the degeneracy of the genetic code.

[0093] The nucleotide sequence encoding the xylose isomerase may encode either a prokaryotic or an eukaryotic xylose isomerase, i.e. a xylose isomerase with an amino acid sequence that is identical to that of a xylose isomerase that naturally occurs in the prokaryotic or eukaryotic organism. The present inventors have found that the ability of a particular xylose isomerase to confer to a eukaryotic host cell the ability to isomerise xylose into xylulose does not depend so much on whether the isomerase is of prokaryotic or eukaryotic origin. Rather this depends on the relatedness of the isomerase's amino acid sequence to that of the Piromyces sequence (SEQ ID NO. 7). Surprisingly, the eukaryotic Piromyces isomerase is more related to prokaryotic isomerases than to other known eukaryotic isomerases. Therefore, a preferred nucleotide sequence encodes a xylose isomerase having an amino acid sequence that is related to the Piromyces sequence as defined above. A preferred nucleotide sequence encodes a fungal xylose isomerase (e.g. from a Basidiomycete), more preferably a xylose isomerase from an anaerobic fungus, e.g. a xylose isomerase from an anaerobic fungus that belongs to the families Neocallimastix, Caecomyces, Piromyces, Orpinomyces, or Ruminomyces. Alternatively, a preferred nucleotide sequence encodes a bacterial xylose isomerase, preferably a Gram-negative bacterium, more preferably an isomerase from the class *Bacteroides*, or from the genus *Bacteroides*, most preferably from *B. thetaiotaomicron* (SEQ ID NO. 15).

[0094] To increase the likelihood that the xylose isomerase is expressed in active form in a eukaryotic host cell such as yeast, the nucleotide sequence encoding the xylose isomerase may be adapted to optimise its codon usage to that of the eukaryotic host cell as earlier defined herein.

[0095] A host cell for transformation with the nucleotide sequence encoding the xylose isomerase as described above, preferably is a host capable of active or passive xylose transport into the cell. The host cell preferably contains active glycolysis. The host cell may further contain an endogenous pentose phosphate pathway and may contain endogenous xylulose kinase activity so that xylulose isomerised from xylose may be metabolised to pyruvate. The host further preferably contains enzymes for conversion of pyruvate to a desired fermentation product such as ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic or a cephalosporin. A preferred host cell is a host cell that is naturally capable of alcoholic fermentation, preferably, anaerobic alcoholic fermentation. The host cell further preferably has a high tolerance to ethanol, a high tolerance to low pH (i.e. capable of growth at a pH lower than 5, 4, 3, or 2.5) and towards organic acids like lactic acid, acetic acid or formic acid and sugar degradation products such as furfural and hydroxy-methylfurfural, and a high tolerance to elevated temperatures. Any of these characteristics or activities of the host cell may be naturally present in the host cell or may be introduced or modified by genetic modification. A suitable cell is a eukaryotic microorganism like e.g. a fungus, however, most suitable as host cell are yeasts or filamentous fungi. Preferred yeasts and filamentous fungi have already been defined herein.

[0096] As used herein the wording host cell has the same meaning as cell.

[0097] The cell of the invention is preferably transformed with a nucleic acid construct comprising the nucleotide sequence encoding the xylose isomerase. The nucleic acid construct that is preferably used is the same as the one used comprising the nucleotide sequence encoding araA, araB or araD.

[0098] In another preferred embodiment of the invention, the cell of the invention:

[0099] expressing araA, araB and araD, and exhibiting the ability to directly isomerise xylose into xylulose, as earlier defined herein

further comprises a genetic modification that increases the flux of the pentose phosphate pathway, as described in WO 06/009434. In particular, the genetic modification causes an increased flux of the non-oxidative part pentose phosphate pathway. A genetic modification that causes an increased flux of the non-oxidative part of the pentose phosphate pathway is herein understood to mean a modification that increases the flux by at least a factor 1.1, 1.2, 1.5, 2, 5, 10 or 20 as compared to the flux in a strain which is genetically identical except for the genetic modification causing the increased flux. The flux of the non-oxidative part of the pentose phosphate pathway may be measured by growing the modified host on xylose as sole carbon source, determining the specific xylose consumption rate and substracting the specific xylitol production rate from the specific xylose consumption rate, if any xylitol is produced. However, the flux of the non-oxidative part of the pentose phosphate pathway is proportional with the growth rate on xylose as sole carbon source, preferably with the anaerobic growth rate on xylose as sole carbon source. There is a linear relation between the growth rate on xylose as sole carbon source (μ_{max}) and the flux of the non-oxidative part of the pentose phosphate pathway. The specific xylose consumption rate (Q_s) is equal to the growth rate (μ) divided by the yield of biomass on sugar (Y_{xs}) because the yield of biomass on sugar is constant (under a given set of Conditions: anaerobic, growth medium, pH, genetic background of the strain, etc.; i.e. $Q_s=\mu/Y_{xs}$). Therefore the increased flux of the non-oxidative part of the pentose phosphate pathway may be deduced from the increase in maximum growth rate under these conditions. In a preferred embodiment, the cell comprises a genetic modification that increases the flux of the pentose phosphate pathway and has a specific xylose consumption rate of at least 346 mg xylose/g biomass/h.

[0100] Genetic modifications that increase the flux of the pentose phosphate pathway may be introduced in the host cell in various ways. These including e.g. achieving higher steady state activity levels of xylulose kinase and/or one or more of the enzymes of the non-oxidative part pentose phosphate pathway and/or a reduced steady state level of unspecific aldose reductase activity. These changes in steady state activity levels may be effected by selection of mutants (spontaneous or induced by chemicals or radiation) and/or by recombinant DNA technology e.g. by overexpression or inactivation, respectively, of genes encoding the enzymes or factors regulating these genes.

[0101] In a more preferred host cell, the genetic modification comprises overexpression of at least one enzyme of the (non-oxidative part) pentose phosphate pathway. Preferably the enzyme is selected from the group consisting of the enzymes encoding for ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transketolase and transaldolase, as described in WO 06/009434.

[0102] Various combinations of enzymes of the (non-oxidative part) pentose phosphate pathway may be overexpressed. E.g. the enzymes that are overexpressed may be at least the enzymes ribulose-5-phosphate isomerase and ribulose-5-phosphate epimerase; or at least the enzymes ribulose-5-phosphate isomerase and transketolase; or at least the enzymes ribulose-5-phosphate isomerase and transaldolase; or at least the enzymes ribulose-5-phosphate epimerase and transketolase; or at least the enzymes ribulose-5-phosphate epimerase and transaldolase; or at least the enzymes transketolase and transaldolase; or at least the enzymes ribulose-5-phosphate epimerase, transketolase and transaldolase; or at least the enzymes ribulose-5-phosphate isomerase, transketolase and transaldolase; or at least the enzymes ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, and transaldolase; or at least the enzymes ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, and transketolase. In one embodiment of the invention each of the enzymes ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transketolase and transaldolase are overexpressed in the host cell. More preferred is a host cell in which the genetic modification comprises at least overexpression of both the enzymes transketolase and transaldolase as such a host cell is already capable of anaerobic growth on xylose. In fact, under some conditions we have found that host cells overexpressing only the transketolase and the transaldolase already have the same anaerobic growth rate on xylose as do host cells that overexpress all four of the enzymes, i.e. the ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transketolase and transaldolase. Moreover, host cells overexpressing both of the enzymes ribulose-5-phosphate isomerase and ribulose-5-phosphate epimerase are preferred over host cells overexpressing only the isomerase or only the epimerase as overexpression of only one of these enzymes may produce metabolic imbalances.

[0103] There are various means available in the art for overexpression of enzymes in the cells of the invention. In particular, an enzyme may be overexpressed by increasing the copy number of the gene coding for the enzyme in the host cell, e.g. by integrating additional copies of the gene in the host cell's genome, by expressing the gene from an episomal multicopy expression vector or by introducing a episomal expression vector that comprises multiple copies of the gene.

[0104] Alternatively overexpression of enzymes in the host cells of the invention may be achieved by using a promoter that is not native to the sequence coding for the enzyme to be overexpressed, i.e. a promoter that is heterologous to the coding sequence to which it is operably linked. Suitable promoters to this end have already been defined herein.

[0105] The coding sequence used for overexpression of the enzymes preferably is homologous to the host cell of the invention. However, coding sequences that are heterologous to the host cell of the invention may likewise be applied, as mentioned in WO 06/009434.

[0106] A nucleotide sequence used for overexpression of ribulose-5-phosphate isomerase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with ribulose-5-phosphate isomerase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 17 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 18, under moderate conditions, preferably under stringent conditions.

[0107] A nucleotide sequence used for overexpression of ribulose-5-phosphate epimerase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with ribulose-5-phosphate epimerase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 19 or whereby the nucleotide sequence is capable of hybridising

with the nucleotide sequence of SEQ ID NO. 20, under moderate conditions, preferably under stringent conditions.

[0108] A nucleotide sequence used for overexpression of transketolase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with transketolase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 21 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 22, under moderate conditions, preferably under stringent conditions.

[0109] A nucleotide sequence used for overexpression of transaldolase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with transaldolase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 23 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 24, under moderate conditions, preferably under stringent conditions.

[0110] Overexpression of an enzyme, when referring to the production of the enzyme in a genetically modified host cell, means that the enzyme is produced at a higher level of specific enzymatic activity as compared to the unmodified host cell under identical conditions. Usually this means that the enzymatically active protein (or proteins in case of multi-subunit enzymes) is produced in greater amounts, or rather at a higher steady state level as compared to the unmodified host cell under identical conditions. Similarly this usually means that the mRNA coding for the enzymatically active protein is produced in greater amounts, or again rather at a higher steady state level as compared to the unmodified host cell under identical conditions. Overexpression of an enzyme is thus preferably determined by measuring the level of the enzyme's specific activity in the host cell using appropriate enzyme assays as described herein. Alternatively, overexpression of the enzyme may determined indirectly by quantifying the specific steady state level of enzyme protein, e.g. using antibodies specific for the enzyme, or by quantifying the specific steady level of the mRNA coding for the enzyme. The latter may particularly be suitable for enzymes of the pentose phosphate pathway for which enzymatic assays are not easily feasible as substrates for the enzymes are not commercially available. Preferably in the host cells of the invention, an enzyme to be overexpressed is overexpressed by at least a factor 1.1, 1.2, 1.5, 2, 5, 10 or 20 as compared to a strain which is genetically identical except for the genetic modification causing the overexpression. It is to be understood that these levels of overexpression may apply to the steady state level of the enzyme's activity, the steady state level of the enzyme's protein as well as to the steady state level of the transcript coding for the enzyme.

[0111] In a further preferred embodiment, the host cell of the invention:

[0112] expressing araA, araB and araD, and exhibiting the ability to directly isomerise xylose into xylulose, and optionally

[0113] comprising a genetic modification that increase the flux of the pentose pathway as earlier defined herein

further comprises a genetic modification that increases the specific xylulose kinase activity. Preferably the genetic modification causes overexpression of a xylulose kinase, e.g. by overexpression of a nucleotide sequence encoding a xylulose kinase. The gene encoding the xylulose kinase may be endogenous to the host cell or may be a xylulose kinase that is heterologous to the host cell. A nucleotide sequence used for overexpression of xylulose kinase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with xylulose kinase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 25 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 26, under moderate conditions, preferably under stringent conditions.

[0114] A particularly preferred xylulose kinase is a xylose kinase that is related to the xylulose kinase xylB from Piromyces as mentioned in WO 03/0624430. A more preferred nucleotide sequence for use in overexpression of xylulose kinase in the host cell of the invention is a nucleotide sequence encoding a polypeptide with xylulose kinase activity, whereby preferably the polypeptide has an amino acid sequence having at least 45, 50, 55, 60, 65, 70, 80, 90 or 95% identity with SEQ ID NO. 27 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 28, under moderate conditions, preferably under stringent conditions.

[0115] In the host cells of the invention, genetic modification that increases the specific xylulose kinase activity may be combined with any of the modifications increasing the flux of the pentose phosphate pathway as described above, but this combination is not essential for the invention. Thus, a host cell of the invention comprising a genetic modification that increases the specific xylulose kinase activity in addition to the expression of the araA, araB and araD enzymes as defined herein is specifically included in the invention. The various means available in the art for achieving and analysing overexpression of a xylulose kinase in the host cells of the invention are the same as described above for enzymes of the pentose phosphate pathway. Preferably in the host cells of the invention, a xylulose kinase to be overexpressed is overexpressed by at least a factor 1.1, 1.2, 1.5, 2, 5, 10 or 20 as compared to a strain which is genetically identical except for the genetic modification causing the overexpression. It is to be understood that these levels of overexpression may apply to the steady state level of the enzyme's activity, the steady state level of the enzyme's protein as well as to the steady state level of the transcript coding for the enzyme.

[0116] In a further preferred embodiment, the host cell of the invention:

[0117] expressing araA, araB and araD, and exhibiting the ability to directly isomerise xylose into xylulose, and optionally

[0118] comprising a genetic modification that increase the flux of the pentose pathway and/or

[0119] further comprising a genetic modification that increases the specific xylulose kinase activity all as earlier defined herein

further comprises a genetic modification that reduces unspecific aldose reductase activity in the host cell. Preferably, unspecific aldose reductase activity is reduced in the host cell by one or more genetic modifications that reduce the expression of or inactivate a gene encoding an unspecific aldose reductase, as described in WO 06/009434. Preferably, the genetic modifications reduce or inactivate the expression of each endogenous copy of a gene encoding an unspecific aldose reductase in the host cell. Host cells may comprise multiple copies of genes encoding unspecific aldose reductases as a result of di-, poly- or aneu-ploidy, and/or the host

cell may contain several different (iso)enzymes with aldose reductase activity that differ in amino acid sequence and that are each encoded by a different gene. Also in such instances preferably the expression of each gene that encodes an unspecific aldose reductase is reduced or inactivated. Preferably, the gene is inactivated by deletion of at least part of the gene or by disruption of the gene, whereby in this context the term gene also includes any non-coding sequence up- or downstream of the coding sequence, the (partial) deletion or inactivation of which results in a reduction of expression of unspecific aldose reductase activity in the host cell. A nucleotide sequence encoding an aldose reductase whose activity is to be reduced in the host cell of the invention is a nucleotide sequence encoding a polypeptide with aldose reductase activity, whereby preferably the polypeptide has an amino acid sequence having at least 50, 60, 70, 80, 90 or 95% identity with SEQ ID NO. 29 or whereby the nucleotide sequence is capable of hybridising with the nucleotide sequence of SEQ ID NO. 30 under moderate conditions, preferably under stringent conditions.

[0120] In the host cells of the invention, the expression of the araA, araB and araD enzymes as defined herein is combined with genetic modification that reduces unspecific aldose reductase activity. The genetic modification leading to the reduction of unspecific aldose reductase activity may be combined with any of the modifications increasing the flux of the pentose phosphate pathway and/or with any of the modifications increasing the specific xylulose kinase activity in the host cells as described above, but these combinations are not essential for the invention. Thus, a host cell expressing araA, araB, and araD, comprising an additional genetic modification that reduces unspecific aldose reductase activity is specifically included in the invention.

[0121] In a preferred embodiment, the host cell is CBS120327 deposited at the CBS Institute (The Netherlands) on Sep. 27, 2006.

[0122] In a further preferred embodiment, the invention relates to modified host cells that are further adapted to L-arabinose (use L-arabinose and/or convert it into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product and optionally xylose utilisation by selection of mutants, either spontaneous or induced (e.g. by radiation or chemicals), for growth on L-arabinose and optionally xylose, preferably on L-arabinose and optionally xylose as sole carbon source, and more preferably under anaerobic conditions. Selection of mutants may be performed by serial passaging of cultures as e.g. described by Kuyper et al. (2004, FEMS Yeast Res. 4: 655-664) and/or by cultivation under selective pressure in a chemostat culture as is described in Example 4 of WO 06/009434. This selection process may be continued as long as necessary. This selection process is preferably carried out during one week till one year. However, the selection process may be carried out for a longer period of time if necessary. During the selection process, the cells are preferably cultured in the presence of approximately 20 g/l L-arabinose and/or approximately 20 g/l xylose. The cell obtained at the end of this selection process is expected to be improved as to its capacities of using L-arabinose and/or xylose, and/or converting L-arabinose into L-ribulose and/or xylulose 5-phosphate and/or a desired fermentation product such as ethanol. In this context "improved cell" may mean that the obtained cell is able to use L-arabinose and/or xylose in a more efficient way than the cell it derives from. For example, the obtained cell is expected to better grow: increase of the specific growth rate of at least 2% than the cell it derives from under the same conditions. Preferably, the increase is of at least 4%, 6%, 8%, 10%, 15%, 20%, 25% or more. The specific growth rate may be calculated from OD_{660} as known to the skilled person. Therefore, by monitoring the OD_{660}, one can deduce the specific growth rate. In this context "improved cell" may also mean that the obtained cell converts L-arabinose into L-ribulose and/or xylulose 5-phosphate and/or a desired fermentation product such as ethanol in a more efficient way than the cell it derives from. For example, the obtained cell is expected to produce higher amounts of L-ribulose and/or xylulose 5-phosphate and/or a desired fermentation product such as ethanol: increase of at least one of these compounds of at least 2% than the cell it derives from under the same conditions. Preferably, the increase is of at least 4%, 6%, 8%, 10%, 15%, 20%, 25% or more. In this context "improved cell" may also mean that the obtained cell converts xylose into xylulose and/or a desired fermentation product such as ethanol in a more efficient way than the cell it derives from. For example, the obtained cell is expected to produce higher amounts of xylulose and/or a desired fermentation product such as ethanol: increase of at least one of these compounds of at least 2% than the cell it derives from under the same conditions. Preferably, the increase is of at least 4%, 6%, 8%, 10%, 15%, 20%, 25% or more.

[0123] In a preferred host cell of the invention at least one of the genetic modifications described above, including modifications obtained by selection of mutants, confer to the host cell the ability to grow on L-arabinose and optionally xylose as carbon source, preferably as sole carbon source, and preferably under anaerobic conditions. Preferably the modified host cell produce essentially no xylitol, e.g. the xylitol produced is below the detection limit or e.g. less than 5, 2, 1, 0.5, or 0.3% of the carbon consumed on a molar basis.

[0124] Preferably the modified host cell has the ability to grow on L-arabinose and optionally xylose as sole carbon source at a rate of at least 0.001, 0.005, 0.01, 0.03, 0.05, 0.1, 0.2, 0.25 or 0.3 h^{-1} under aerobic conditions, or, if applicable, at a rate of at least 0.001, 0.005, 0.01, 0.03, 0.05, 0.07, 0.08, 0.09, 0.1, 0.12, 0.15 or 0.2 h^{-1} under anaerobic conditions Preferably the modified host cell has the ability to grow on a mixture of glucose and L-arabinose and optionally xylose (in a 1:1 weight ratio) as sole carbon source at a rate of at least 0.001, 0.005, 0.01, 0.03, 0.05, 0.1, 0.2, 0.25 or 0.3 h^{-1} under aerobic conditions, or, if applicable, at a rate of at least 0.001, 0.005, 0.01, 0.03, 0.05, 0.1, 0.12, 0.15, or 0.2 h^{-1} under anaerobic conditions.

[0125] Preferably, the modified host cell has a specific L-arabinose and optionally xylose consumption rate of at least 346, 350, 400, 500, 600, 650, 700, 750, 800, 900 or 1000 mg/g cells/h. Preferably, the modified host cell has a yield of fermentation product (such as ethanol) on L-arabinose and optionally xylose that is at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 85, 90, 95 or 98% of the host cell's yield of fermentation product (such as ethanol) on glucose. More preferably, the modified host cell's yield of fermentation product (such as ethanol) on L-arabinose and optionally xylose is equal to the host cell's yield of fermentation product (such as ethanol) on glucose. Likewise, the modified host cell's biomass yield on L-arabinose and optionally xylose is preferably at least 55, 60, 70, 80, 85, 90, 95 or 98% of the host cell's biomass yield on glucose. More preferably, the modified host cell's biomass yield on L-arabinose and optionally xylose is equal to the host cell's biomass yield on glucose. It

is understood that in the comparison of yields on glucose and L-arabinose and optionally xylose both yields are compared under aerobic conditions or both under anaerobic conditions.

[0126] In a more preferred embodiment, the host cell is CBS120328 deposited at the CBS Institute (The Netherlands) on Sep. 27, 2006 or CBS121879 deposited at the CBS Institute (The Netherlands) on Sep. 20, 2007.

[0127] In a preferred embodiment, the cell expresses one or more enzymes that confer to the cell the ability to produce at least one fermentation product selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin. In a more preferred embodiment, the host cell of the invention is a host cell for the production of ethanol. In another preferred embodiment, the invention relates to a transformed host cell for the production of fermentation products other than ethanol. Such non-ethanolic fermentation products include in principle any bulk or fine chemical that is producible by a eukaryotic microorganism such as a yeast or a filamentous fungus. Such fermentation products include e.g. lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin. A preferred host cell of the invention for production of non-ethanolic fermentation products is a host cell that contains a genetic modification that results in decreased alcohol dehydrogenase activity.

Method

[0128] In a further aspect, the invention relates to fermentation processes in which a host cell of the invention is used for the fermentation of a carbon source comprising a source of L-arabinose and optionally a source of xylose. Preferably, the source of L-arabinose and the source of xylose are L-arabinose and xylose. In addition, the carbon source in the fermentation medium may also comprise a source of glucose. The source of L-arabinose, xylose or glucose may be L-arabinose, xylose or glucose as such or may be any carbohydrate oligo- or polymer comprising L-arabinose, xylose or glucose units, such as e.g. lignocellulose, xylans, cellulose, starch, arabinan and the like. For release of xylose or glucose units from such carbohydrates, appropriate carbohydrases (such as xylanases, glucanases, amylases and the like) may be added to the fermentation medium or may be produced by the modified host cell. In the latter case the modified host cell may be genetically engineered to produce and excrete such carbohydrases. An additional advantage of using oligo- or polymeric sources of glucose is that it enables to maintain a low(er) concentration of free glucose during the fermentation, e.g. by using rate-limiting amounts of the carbohydrases. This, in turn, will prevent repression of systems required for metabolism and transport of non-glucose sugars such as xylose. In a preferred process the modified host cell ferments both the L-arabinose (optionally xylose) and glucose, preferably simultaneously in which case preferably a modified host cell is used which is insensitive to glucose repression to prevent diauxic growth. In addition to a source of L-arabinose, optionally xylose (and glucose) as carbon source, the fermentation medium will further comprise the appropriate ingredient required for growth of the modified host cell. Compositions of fermentation media for growth of microorganisms such as yeasts or filamentous fungi are well known in the art.

[0129] In a preferred process, there is provided a process for producing a fermentation product selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin whereby the process comprises the steps of:

[0130] (a) fermenting a medium containing a source of L-arabinose and optionally xylose with a modified host cell as defined herein, whereby the host cell ferments L-arabinose and optionally xylose to the fermentation product, and optionally,

[0131] (b) recovering the fermentation product.

[0132] The fermentation process is a process for the production of a fermentation product such as e.g. ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic, such as Penicillin G or Penicillin V and fermentative derivatives thereof, and/or a cephalosporin. The fermentation process may be an aerobic or an anaerobic fermentation process. An anaerobic fermentation process is herein defined as a fermentation process run in the absence of oxygen or in which substantially no oxygen is consumed, preferably less than 5, 2.5 or 1 mmol/L/h, more preferably 0 mmol/L/h is consumed (i.e. oxygen consumption is not detectable), and wherein organic molecules serve as both electron donor and electron acceptors. In the absence of oxygen, NADH produced in glycolysis and biomass formation, cannot be oxidised by oxidative phosphorylation. To solve this problem many microorganisms use pyruvate or one of its derivatives as an electron and hydrogen acceptor thereby regenerating NAD^+. Thus, in a preferred anaerobic fermentation process pyruvate is used as an electron (and hydrogen acceptor) and is reduced to fermentation products such as ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotics and a cephalosporin. In a preferred embodiment, the fermentation process is anaerobic. An anaerobic process is advantageous since it is cheaper than aerobic processes: less special equipment is needed. Furthermore, anaerobic processes are expected to give a higher product yield than aerobic processes. Under aerobic conditions, usually the biomass yield is higher than under anaerobic conditions. As a consequence, usually under aerobic conditions, the expected product yield is lower than under anaerobic conditions. According to the inventors, the process of the invention is the first anaerobic fermentation process with a medium comprising a source of L-arabinose that has been developed so far.

[0133] In another preferred embodiment, the fermentation process is under oxygen-limited conditions. More preferably, the fermentation process is aerobic and under oxygen-limited conditions. An oxygen-limited fermentation process is a process in which the oxygen consumption is limited by the oxygen transfer from the gas to the liquid. The degree of oxygen limitation is determined by the amount and composition of the ingoing gasflow as well as the actual mixing/mass transfer properties of the fermentation equipment used. Preferably, in a process under oxygen-limited conditions, the rate of oxygen consumption is at least 5.5, more preferably at least 6 and even more preferably at least 7 mmol/L/h.

[0134] The fermentation process is preferably run at a temperature that is optimal for the modified cell. Thus, for most

yeasts or fungal cells, the fermentation process is performed at a temperature which is less than 42° C., preferably less than 38° C. For yeast or filamentous fungal host cells, the fermentation process is preferably performed at a temperature which is lower than 35, 33, 30 or 28° C. and at a temperature which is higher than 20, 22, or 25° C.

[0135] A preferred process is a process for the production of ethanol, whereby the process comprises the steps of: (a) fermenting a medium containing a source of L-arabinose and optionally xylose with a modified host cell as defined herein, whereby the host cell ferments L-arabinose and optionally xylose to ethanol; and optionally, (b) recovery of the ethanol. The fermentation medium may also comprise a source of glucose that is also fermented to ethanol. In a preferred embodiment, the fermentation process for the production of ethanol is anaerobic. Anaerobic has already been defined earlier herein. In another preferred embodiment, the fermentation process for the production of ethanol is aerobic. In another preferred embodiment, the fermentation process for the production of ethanol is under oxygen-limited conditions, more preferably aerobic and under oxygen-limited conditions. Oxygen-limited conditions have already been defined earlier herein.

[0136] In the process, the volumetric ethanol productivity is preferably at least 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0 or 10.0 g ethanol per litre per hour. The ethanol yield on L-arabinose and optionally xylose and/or glucose in the process preferably is at least 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95 or 98%. The ethanol yield is herein defined as a percentage of the theoretical maximum yield, which, for glucose and L-arabinose and optionally xylose is 0.51 g. ethanol per g. glucose or xylose. In another preferred embodiment, the invention relates to a process for producing a fermentation product selected from the group consisting of lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin. The process preferably comprises the steps of (a) fermenting a medium containing a source of L-arabinose and optionally xylose with a modified host cell as defined herein above, whereby the host cell ferments L-arabinose and optionally xylose to the fermentation product, and optionally, (b) recovery of the fermentation product. In a preferred process, the medium also contains a source of glucose.

[0137] In the fermentation process of the invention leading to the production of ethanol, several advantages can be cited by comparison to known ethanol fermentations processes:

[0138] anaerobic processes are possible.

[0139] oxygen limited conditions are also possible.

[0140] higher ethanol yields and ethanol production rates can be obtained.

[0141] the strain used may be able to use L-arabinose and optionally xylose.

[0142] Alternatively to the fermentation processes described above, another fermentation process is provided as a further aspect of the invention wherein, at least two distinct cells are used for the fermentation of a carbon source comprising at least two sources of carbon selected from the group consisting of but not limited thereto: a source of L-arabinose, a source of xylose and a source of glucose. In this fermentation process, "at least two distinct cells" means this process is preferably a co-fermentation process. In one preferred embodiment, two distinct cells are used: one being the one of the invention as earlier defined able to use L-arabinose, and/or to convert it into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product such as ethanol and optionally being able to use xylose, the other one being for example a strain which is able to use xylose and/or convert it into a desired fermentation product such as ethanol as defined in WO 03/062430 and/or WO 06/009434. A cell which is able to use xylose is preferably a strain which exhibits the ability of directly isomerising xylose into xylulose (in one step) as earlier defined herein. These two distinct strains are preferably cultived in the presence of a source of L-arabinose, a source of xylose and optionally a source of glucose. Three distinct cells or more may be co-cultivated and/or three or more sources of carbon may be used, provided at least one cell is able to use at least one source of carbon present and/or to convert it into a desired fermentation product such as ethanol. The expression "use at least one source of carbon" has the same meaning as the expression "use of L-arabinose". The expression "convert it (i.e. a source of carbon) into a desired fermentation product has the same meaning as the expression "convert L-arabinose into a desired fermentation product".

[0143] In a preferred embodiment, the invention relates to a process for producing a fermentation product selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, amino acids, 1,3-propane-diol, ethylene, glycerol, butanol, β-lactam antibiotics and cephalosporins, whereby the process comprises the steps of:

[0144] (a) fermenting a medium containing at least a source of L-arabinose and a source of xylose with a cell of the invention as earlier defined herein and a cell able to use xylose and/or exhibiting the ability to directly isomerise xylose into xylulose, whereby each cell ferments L-arabinose and/or xylose to the fermentation product, and optionally,

[0145] (b) recovering the fermentation product.

All preferred embodiments of the fermentation processes as described above are also preferred embodiments of this further fermentation processes: identity of the fermentation product, identity of source of L-arabinose and source of xylose, conditions of fermentation (aerobical or anaerobical conditions, oxygen-limited conditions, temperature at which the process is being carried out, productivity of ethanol, yield of ethanol).

Genetic Modifications

[0146] For overexpression of enzymes in the host cells of the inventions as described above, as well as for additional genetic modification of host cells, preferably yeasts, host cells are transformed with the various nucleic acid constructs of the invention by methods well known in the art. Such methods are e.g. known from standard handbooks, such as Sambrook and Russel (2001) "Molecular Cloning: A Laboratory Manual (3rd edition), Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, or F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987). Methods for transformation and genetic modification of fungal host cells are known from e.g. EP-A-0 635 574, WO 98/46772, WO 99/60102 and WO 00/37671.

[0147] Promoters for use in the nucleic acid constructs for overexpression of enzymes in the host cells of the invention have been described above. In the nucleic acid constructs for overexpression, the 3'-end of the nucleotide acid sequence

encoding the enzyme(s) preferably is operably linked to a transcription terminator sequence. Preferably the terminator sequence is operable in a host cell of choice, such as e.g. the yeast species of choice. In any case the choice of the terminator is not critical; it may e.g. be from any yeast gene, although terminators may sometimes work if from a non-yeast, eukaryotic, gene. The transcription termination sequence further preferably comprises a polyadenylation signal. Preferred terminator sequences are the alcohol dehydrogenase (ADH1) and the PGI1 terminators. More preferably, the ADH1 and the PGI1 terminators are both from *S. cerevisiae* (SEQ ID NO:50 and SEQ ID NO:53 respectively).

[0148] Optionally, a selectable marker may be present in the nucleic acid construct. As used herein, the term "marker" refers to a gene encoding a trait or a phenotype which permits the selection of, or the screening for, a host cell containing the marker. The marker gene may be an antibiotic resistance gene whereby the appropriate antibiotic can be used to select for transformed cells from among cells that are not transformed. Preferably however, non-antibiotic resistance markers are used, such as auxotrophic markers (URA3, TRP1, LEU). In a preferred embodiment the host cells transformed with the nucleic acid constructs are marker gene free. Methods for constructing recombinant marker gene free microbial host cells are disclosed in EP-A-0 635 574 and are based on the use of bidirectional markers. Alternatively, a screenable marker such as Green Fluorescent Protein, lacZ, luciferase, chloramphenicol acetyltransferase, beta-glucuronidase may be incorporated into the nucleic acid constructs of the invention allowing to screen for transformed cells.

[0149] Optional further elements that may be present in the nucleic acid constructs of the invention include, but are not limited to, one or more leader sequences, enhancers, integration factors, and/or reporter genes, intron sequences, centromers, telomers and/or matrix attachment (MAR) sequences. The nucleic acid constructs of the invention may further comprise a sequence for autonomous replication, such as an ARS sequence. Suitable episomal nucleic acid constructs may e.g. be based on the yeast 2μ or pKD1 (Fleer et al., 1991, Biotechnology 9:968-975) plasmids. Alternatively the nucleic acid construct may comprise sequences for integration, preferably by homologous recombination. Such sequences may thus be sequences homologous to the target site for integration in the host cell's genome. The nucleic acid constructs of the invention can be provided in a manner known per se, which generally involves techniques such as restricting and linking nucleic acids/nucleic acid sequences, for which reference is made to the standard handbooks, such as Sambrook and Russel (2001) "Molecular Cloning: A Laboratory Manual (3rd edition), Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press.

[0150] Methods for inactivation and gene disruption in yeast or fungi are well known in the art (see e.g. Fincham, 1989, Microbiol Rev. 53(1):148-70 and EP-A-0 635 574).

[0151] In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

[0152] The invention is further described by the following examples, which should not be construed as limiting the scope of the invention.

EXAMPLES

Plasmid and Strain Construction

Strains

[0153] The L-arabinose consuming *Sachharomyces cerevisiae* strain described in this work is based on strain RWB220, which is itself a derivative of RWB217. RWB217 is a CEN. PK strain in which four genes coding for the expression of enzymes in the pentose phosphate pathway have been overexpressed, TAL1, TKL1, RPE1, RKI1 (Kuyper et al., 2005a). In addition the gene coding for an aldose reductase (GRE3), has been deleted. Strain RWB217 also contains two plasmids, a single copy plasmid with a LEU2 marker for overexpression of the xylulokinase (XKS1) and an episomal, multicopy plasmid with URA3 as the marker for the expression of the xylose isomerase, XylA. RWB217 was subjected to a selection procedure for improved growth on xylose which is described in Kuyper et al. (2005b). The procedure resulted in two pure strains, RWB218 (Kuyper et al., 2005b) and RWB219. The difference between RWB218 and RWB219 is that after the selection procedure, RWB218 was obtained by plating and restreaking on mineral medium with glucose as the carbon source, while for RWB219 xylose was used.

[0154] Strain RWB219 was grown non-selectively on YP with glucose (YPD) as the carbon source in order to facilitate the loss of both plasmids. After plating on YPD single colonies were tested for plasmid loss by looking at uracil and leucine auxotrophy. A strain that had lost both plasmids was transformed with pSH47, containing the cre recombinase, in order to remove a KanMX cassette (Guldener et al., 1996), still present after integrating the RKI1 overexpression construct. Colonies with the plasmid were resuspended in Yeast Peptone medium (YP) (10 g/l yeast extract and 20 g/l peptone both from BD Difco Belgium) with 1% galactose and incubated for 1 hour at 30° C. About 200 cells were plated on YPD. The resulting colonies were checked for loss of the KanMX marker (G418 resistance) and pSH47 (URA3). A strain that had lost both the KanMX marker and the pSH47 plasmid was then named RWB220. To obtain the strain tested in this patent, RWB220 was transformed with pRW231 and pRW243 (table 2), resulting in strain IMS0001.

[0155] During construction strains were maintained on complex YP: 10 g l^{-1} yeast extract (BD Difco), 20 g l^{-1} peptone (BD Difco) or synthetic medium (MY) (Verduyn et al., 1992) supplemented with glucose (2%) as carbon source (YPD or MYD) and 1.5% agar in the case of plates. After transformation with plasmids strains were plated on MYD. Transformations of yeast were done according to Gietz and Woods (2002). Plasmids were amplified in *Escherichia coli* strain XL-1 blue (Stratagene, La Jolla, Calif., USA). Transformation was performed according to Inoue et al. (1990). *E. coli* was grown on LB (Luria-Bertani) plates or in liquid TB (Terrific Broth) medium for the isolation of plasmids (Sambrook et al, 1989).

Plasmids

[0156] In order to grow on L-arabinose, yeast needs to express three different genes, an L-arabinose isomerase (AraA), a L-ribulokinase (AraB), and a L-ribulose-5-P 4-epi-

merase (AraD) (Becker and Boles, 2003). In this work we have chosen to express AraA, AraB, and AraD from the lactic acid bacterium *Lactobacillus plantarum* in *S. cerevisiae*. Because the eventual aim is to consume L-arabinose in combination with other sugars, like D-xylose, the genes encoding the bacterial L-arabinose pathway were combined on the same plasmid with the genes coding for D-xylose consumption.

[0157] In order to get a high level of expression, the *L. plantarum* AraA and AraD genes were ligated into plasmid pAKX002, the 2μ XylA bearing plasmid.

[0158] The AraA cassette was constructed by amplifying a truncated version of the TDH3 promoter with SpeI5'Ptdh3 and 5'AraAPtdh3 (SEQ ID NO: 49), the AraA gene with Ptdh5'AraA and Tadh3'AraA and the ADH1 terminator (SEQ ID NO:50) with 3'AraATadh1 and 3'Tadh1-SpeI. The three fragments were extracted from gel and mixed in roughly equimolar amounts. On this mixture a PCR was performed using the SpeI-5'Ptdh3 and 3'Tadh1SpeI oligos. The resulting P_{TDH3}-AraA-T_{ADH1} cassette was gel purified, cut at the 5' and **3'** SpeI sites and then ligated into pAKX002 cut with NheI, resulting in plasmid pRW230.

[0159] The AraD construct was made by first amplifying a truncated version of the HXT7 promoter (SEQ ID NO:52) with oligos SalI5'Phxt7 and 5'AraDPhxt, the AraD gene with Phxt5'AraD and Tpgi3'AraD and the GPI1 terminator (SEQ ID NO:53) region with the 3'AraDTpgi and 3'TpgiSalI oligos. The resulting fragments were extracted from gel and mixed in roughly equimolar amounts, after which a PCR was performed using the SalI5'Phxt7 and 3'Tpgi1SalI oligos. The resulting P_{HXT7}-AraD-T_{PGI1} cassette was gel purified, cut at the 5' and 3' SalI sites and then ligated into pRW230 cut with XhoI, resulting in plasmid pRW231 (FIG. **1**).

[0160] Since too high an expression of the L-ribulokinase is detrimental to growth (Becker and Boles, 2003), the AraB gene was combined with the XKS1 gene, coding for xylulokinase, on an integration plasmid. For this, p415ADHXKS (Kuyper et al., 2005a) was first changed into pRW229, by cutting both p415ADHXKS and pRS305 with PvuI and ligating the ADHXKS-containing PvuI fragment from p415ADHXKS to the vector backbone from pRS305, resulting in pRW229.

[0161] A cassette, containing the *L. plantarum* AraB gene between the PGI1 promoter (SEQ ID NO:51) and ADH1 terminator (SEQ ID NO:50) was made by amplifying the PGI1 promoter with the SacI5'Ppgi1 and 5'AraBPpgi1 oligos, the AraB gene with the Ppgi5'AraB and Tadh3'AraB oligos and the ADH1 terminator with 3'AraBTadh1 and 3'Tadh1SacI oligos. The three fragments were extracted from gel and mixed in roughly equimolar amounts. On this mixture a PCR was performed using the SacI-5'Ppgi 1 and 3'Tadh1SacI oligos. The resulting P_{PGI1}-AraB-T_{ADH1} cassette was gel purified, cut at the 5' and **3'** Sad sites and then ligated into pRW229 cut with SacI, resulting in plasmid pRW243 (FIG. **1**).

[0162] Strain RWB220 was transformed with pRW231 and pRW243 (table 2), resulting in strain IMS0001.

[0163] Restriction endonucleases (New England Biolabs, Beverly, Mass., USA and Roche, Basel, Switzerland) and DNA ligase (Roche) were used according to the manufacturers' specifications. Plasmid isolation from *E. coli* was performed with the Qiaprep spin miniprep kit (Qiagen, Hilden, Germany). DNA fragments were separated on a 1% agarose (Sigma, St. Louis, Mo., USA) gel in 1×TBE (Sambrook et al, 1989). Isolation of fragments from gel was carried out with the Qiaquick gel extraction kit (Quiagen). Amplification of the (elements of the) AraA, AraB and AraD cassettes was done with $Vent_R$ DNA polymerase (New England Biolabs) according to the manufacturer's specification. The template was chromosomal DNA of *S. cerevisiae* CEN.PK113-7D for the promoters and terminators, or *Lactobacillus plantarum* DSM20205 for the Ara genes. The polymerase chain reaction (PCR) was performed in a Biometra TGradient Thermocycler (Biometra, Gottingen, Germany) with the following settings: 30 cycles of 1 min annealing at 55° C., 60° C. or 65° C., 1 to 3 min extension at 75° C., depending on expected fragment size, and 1 min denaturing at 94° C.

Cultivation and Media

[0164] Shake-flask cultivations were performed at 30° C. in a synthetic medium (Verduyn et al., 1992). The pH of the medium was adjusted to 6.0 with 2 M KOH prior to sterilisation. For solid synthetic medium, 1.5% of agar was added.

[0165] Pre-cultures were prepared by inoculating 100 ml medium containing the appropriate sugar in a 500-ml shake flask with a frozen stock culture. After incubation at 30° C. in an orbital shaker (200 rpm), this culture was used to inoculate either shake-flask cultures or fermenter cultures. The synthetic medium for anaerobic cultivation was supplemented with 0.01 g l^{-1} ergosterol and 0.42 g Tween 80 dissolved in ethanol (Andreasen and Stier, 1953; Andreasen and Stier, 1954). Anaerobic (sequencing) batch cultivation was carried out at 30° C. in 2-1 laboratory fermenters (Applikon, Schiedam, The Netherlands) with a working volume of 1 l. The culture pH was maintained at pH 5.0 by automatic addition of 2 M KOH. Cultures were stirred at 800 rpm and sparged with 0.5 l min^{-1} nitrogen gas (<10 ppm oxygen). To minimise diffusion of oxygen, fermenters were equipped with Norprene tubing (Cole Palmer Instrument company, Vernon Hills, USA). Dissolved oxygen was monitored with an oxygen electrode (Applisens, Schiedam, The Netherlands). Oxygen-limited conditions were achieved in the same experimental set-up by headspace aeration at approximately 0.05 l min^{-1}.

Determination of Dry Weight

[0166] Culture samples (10.0 ml) were filtered over preweighed nitrocellulose filters (pore size 0.45 μm; Gelman laboratory, Ann Arbor, USA). After removal of medium, the filters were washed with demineralised water and dried in a microwave oven (Bosch, Stuttgart, Germany) for 20 min at 360 W and weighed. Duplicate determinations varied by less than 1%.

Gas Analysis

[0167] Exhaust gas was cooled in a condensor (2° C.) and dried with a Permapure dryer type MD-110-48P-4 (Permapure, Toms River, USA). O2 and CO2 concentrations were determined with a NGA 2000 analyser (Rosemount Analytical, Orrville, USA). Exhaust gasflow rate and specific oxygen-consumption and carbondioxide production rates were determined as described previously (Van Urk et al., 1988; Weusthuis et al., 1994). In calculating these biomass-specific rates, volume changes caused by withdrawing culture samples were taken account for.

Metabolite Analysis

[0168] Glucose, xylose, arabinose, xylitol, organic acids, glycerol and ethanol were analysed by HPLC using a Waters

Alliance 2690 HPLC (Waters, Milford, USA) supplied with a BioRad HPX 87H column (BioRad, Hercules, USA), a Waters 2410 refractive-index detector and aWaters 2487 UV detector. The column was eluted at 60° C. with 0.5 g l^{-1} sulphuric acid at a flow rate of 0.6 ml min^{-1}.

Assay for Xylulose 5-Phosphate (Zaldivar J., et al, Appl. Microbiol. Biotechnol., (2002), 59:436-442)

[0169] For the analysis of intracellular metabolites such as xylulose 5-phosphate, 5 ml broth was harvested in duplicate from the reactors, before glucose exhaustion (at 22 and 26 h of cultivation) and after glucose exhaustion (42, 79 and 131 h of cultivation). Procedures for metabolic arrest, solid-phase extraction of metabolites and analysis have been described in detail by Smits H. P. et al. (Anal. Biochem., 261:36-42, (1998)). However, the analysis by high-pressure ion exchange chromatography coupled to pulsed amperometric detection used to analyze cell extracts, was slightly modified. Solutions used were eluent A, 75 mM NaOH, and eluent B, 500 mM NaAc. To prevent contamination of carbonate in the eluent solutions, a 50% NaOH solution with low carbonate concentration (Baker Analysed, Deventer, The Netherlands) was used instead of NaOH pellets. The eluents were degassed with Helium (He) for 30 min and then kept under a He atmosphere. The gradient pump was programmed to generate the following gradients: 100% A and 0% B (0 min), a linear decrease of A to 70% and a linear increase of B to 30% (0-30 min), a linear decrease of A to 30% and a linear increase of B to 70% (30-70 min), a linear decrease of A to 0% and a linear increase of B to 100% (70-75 min), 0% A and 100% B (75-85 min), a linear increase of A to 100% and a linear decrease of B to 0% (85-95 min). The mobile phase was run at a flow rate of 1 ml/min. Other conditions were according to Smits et al. (1998).

Carbon Recovery

[0170] Carbon recoveries were calculated as carbon in products formed, divided by the total amount of sugar carbon consumed, and were based on a carbon content of biomass of 48%. To correct for ethanol evaporation during the fermentations, the amount of ethanol produced was assumed to be equal to the measured cumulative production of CO_2 minus the CO_2 production that occurred due to biomass synthesis (5.85 mmol CO_2 per gram biomass (Verduyn et al., 1990)) and the CO_2 associated with acetate formation.

Selection for Growth on L-Arabinose

[0171] Strain IMS0001 (CBS120327 deposited at the CBS on 27/09/06), containing the genes encoding the pathways for both xylose (XylA and XKS1) and arabinose (AraA, AraB, AraD) metabolization, was constructed according the procedure described above. Although capable of growing on xylose (data not shown), strain IMS0001 did not seem to be capable of growing on solid synthetic medium supplemented with 2% L-arabinose. Mutants of IMS0001 capable of utilizing L-arabinose as carbon source for growth were selected by serial transfer in shake flasks and by sequencing-batch cultivation in fermenters (SBR).

[0172] For the serial transfer experiments, a 500-ml shake flask containing 100 ml synthetic medium containing 0.5% galactose were inoculated with either strain IMS0001, or the reference strain RWB219. After 72 hours, at an optical density at 660 nm of 3.0, the cultures were used to inoculate a new shake flask containing 0.1% galactose and 2% arabinose. Based on HPLC determination with D-ribulose as calibration standard, it was determined that already in the first cultivations of strain IMS0001, on medium containing a galactose/arabinose mixture, part of the arabinose was converted into ribulose and subsequently excreted to the supernatant. These HPLC analyses were performed using a Waters Alliance 2690 HPLC (Waters, Milford, USA) supplied with a BioRad HPX 87H column (BioRad, Hercules, USA), a Waters 2410 refractive-index detector and a Waters 2487 UV detector. The column was eluted at 60° C. with 0.5 g sulphuric acid at a flow rate of 0.6 ml min^{-1}. In contrast to the reference strain RWB219, the OD_{660} of the culture of strain IMS0001 increased after depletion of the galactose. When after approximately 850 hours growth on arabinose by strain IMS0001 was observed (FIG. **2**), this culture was transferred at an OD_{660} of 1.7 to a shake flask containing 2% arabinose. Cultures were then sequentially transferred to fresh medium containing 2% arabinose at an OD_{660} of 2-3. Utilization of arabinose was confirmed by occasionally measuring arabinose concentrations by HPLC (data not shown). The growth rate of these cultures increased from 0 to 0.15 h^{-1} in approximately 3600 hours (FIG. **3**).

[0173] A batch fermentation under oxygen limited conditions was started by inoculating 1 l of synthetic medium supplemented with 2% of arabinose with a 100 ml shake flask culture of arabinose-grown IMS0001 cells with a maximum growth rate on 2% of L-arabinose of approximately 0.12 h^{-1}. When growth on arabinose was observed, the culture was subjected to anaerobic conditions by sparging with nitrogen gas. The sequential cycles of anaerobic batch cultivation were started by either manual or automated replacement of 90% of the culture with synthetic medium with 20 g l^{-1} arabinose. For each cycle during the SBR fermentation, the exponential growth rate was estimated from the CO_2 profile (FIG. **4**). In 13 cycles, the exponential growth rate increased from 0.025 to 0.08 h^{-1}. After 20 cycles a sample was taken, and plated on solid synthetic medium supplemented with 2% of L-arabinose and incubated at 30° C. for several days. Separate colonies were re-streaked twice on solid synthetic medium with L-arabinose. Finally, a shake flask containing synthetic medium with 2% of L-L-arabinose was inoculated with a single colony, and incubated for 5 days at 30° C. This culture was designated as strain IMS0002 (CBS120328 deposited at the Centraal Bureau voor Schimmelculturen (CBS) on 27/09/06). Culture samples were taken, 30% of glycerol was added and samples were stored at −80° C.

Mixed Culture Fermentation

[0174] Biomass hydrolysates, a desired feedstock for industrial biotechnology, contain complex mixtures consisting of various sugars amongst which glucose, xylose and arabinose are commonly present in significant fractions. To accomplish ethanolic fermentation of not only glucose and arabinose, but also xylose, an anaerobic batch fermentation was performed with a mixed culture of the arabinose-fermenting strain IMS0002, and the xylose-fermenting strain RWB218. An anaerobic batch fermenter containing 800 ml of synthetic medium supplied with 30 g l^{-1} D-glucose, 15 g l^{-1} D-xylose, and 15 g l^{-1} L-arabinose was inoculated with 100 ml of pre-culture of strain IMS0002. After 10 hours, a 100 ml inoculum of RWB218 was added. In contrast to the mixed sugar fermentation with only strain IMS0002, both xylose and arabinose were consumed after glucose depletion (FIG. **5**D). The mixed culture completely consumed all sugars, and

within 80 hours 564.0±6.3 mmol l^1 ethanol (calculated from the CO_2 production) was produced with a high overall yield of 0.42 g g^{-1} sugar. Xylitol was produced only in small amounts, to a concentration of 4.7 mmol l^{-1}.

Characterization of Strain IMS0002

[0175] Growth and product formation of strain IMS0002 was determined during anaerobic batch fermentations on synthetic medium with either L-arabinose as the sole carbon source, or a mixture of glucose, xylose and L-arabinose. The pre-cultures for these anaerobic batch fermentations were prepared in shake flasks containing 100 ml of synthetic medium with 2% L-arabinose, by inoculating with −80° C. frozen stocks of strain IMS0002, and incubating for 48 hours at 30° C.

[0176] FIG. **5**A shows that strain IMS0002 is capable of fermenting 20 g l^{-1} L-arabinose to ethanol during an anaerobic batch fermentation of approximately 70 hours. The specific growth rate under anaerobic conditions with L-arabinose as sole carbon source was 0.05±0.001 h^{-1}. Taking into account the ethanol evaporation during the batch fermentation, the ethanol yield from 20 g l^{-1} arabinose was 0.43±0.003 g g^{-1}. Without evaporation correction the ethanol yield was 0.35±0.01 g g^{-1} of arabinose. No formation of arabinitol was observed during anaerobic growth on arabinose. In FIG. **5**B, the ethanolic fermentation of a mixture of 20 g l^{-1} glucose and 20 g l^{-1} L-arabinose by strain IMS0002 is shown. L-arabinose consumption started after glucose depletion. Within 70 hours, both the glucose and L-arabinose were completely consumed. The ethanol yield from the total of sugars was 0.42±0.003 g g^{-1}.

[0177] In FIG. **5**C, the fermentation profile of a mixture of 30 g l^{-1} glucose, 15 g l^{-1} D-xylose, and 15 g l^{-1} L-arabinose by strain IMS0002 is shown. Arabinose consumption started after glucose depletion. Within 80 hours, both the glucose and arabinose were completely consumed. Only 20 mM from 100 mM of xylose was consumed by strain IMS0002. In addition, the formation of 20 mM of xylitol was observed. Apparently, the xylose was converted into xylitol by strain IMS0002. Hence, the ethanol yield from the total of sugars was lower than for the above described fermentations: 0.38±0.001 g g^{-1}. The ethanol yield from the total of glucose and arabinose was similar to the other fermentations: 0.43±0.001 g g^{-1}.

[0178] Table 1 shows the arabinose consumption rates and the ethanol production rates observed for the anaerobic batch fermentation of strain IMS0002. Arabinose was consumed with a rate of 0.23-0.75 g h^{-1} g^{-1} biomass dry weight. The rate of ethanol produced from arabinose varied from 0.08-0.31 g h^{-1} g^{-1} biomass dry weight.

[0179] Initially, the constructed strain IMS0001 was able to ferment xylose (data not shown). In contrast to our expectations, the selected strain IMS0002 was not capable of fermenting xylose to ethanol (FIG. **5**C). To regain the capability of fermenting xylose, a colony of strain IMS0002 was transferred to solid synthetic medium with 2% of D-xylose, and incubated in an anaerobic jar at 30° C. for 25 days. Subsequently, a colony was again transferred to solid synthetic medium with 2% of arabinose. After 4 days of incubation at 30° C., a colony was transferred to a shake flask containing synthetic medium with 2% arabinose. After incubation at 30° C. for 6 days, 30% of glycerol was added, samples were taken and stored at −80° C. A shake flask containing 100 ml of synthetic medium with 2% arabinose was inoculated with such a frozen stock, and was used as preculture for an anaerobic batch fermentation on synthetic medium with 20 g l^{-1} xylose and 20 g l^{-1} arabinose. In FIG. **6**, the fermentation profile of this batch fermentation is shown. Xylose and arabinose were consumed simultaneously. The arabinose was completed within 70 hours, whereas the xylose was completely consumed in 120 hours. At least 250 mM of ethanol was produced from the total of sugars, not taking into account the evaporation of the ethanol. Assuming an end biomass dry weight of 3.2 g l^{-1} (assuming a biomass yield of 0.08 g g^{-1} sugar), the end ethanol concentration estimated from the cumulative CO_2 production (355 mmol l^{-1}) was approximately 330 mmol l^{-1}, corresponding to a ethanol yield of 0.41 g g^{-1} pentose sugar. In addition to ethanol, glycerol, and organic acids, a small amount of xylitol was produced (approximately 5 mM).

Selection of Strain IMS0003

[0180] Initially, the constructed strain IMS0001 was able to ferment xylose (data not shown). In contrast to our expectations, the selected strain IMS0002 was not capable of fermenting xylose to ethanol (FIG. **5**C). To regain the capability of fermenting xylose, a colony of strain IMS0002 was transferred to solid synthetic medium with 2% of D-xylose, and incubated in an anaerobic jar at 30° C. for 25 days. Subsequently, a colony was again transferred to solid synthetic medium with 2% of arabinose. After 4 days of incubation at 30° C., a colony was transferred to a shake flask containing synthetic medium with 2% arabinose. After incubation at 30° C. for 6 days, 30% of glycerol was added, samples were taken and stored at −80° C.

[0181] From this frozen stock, samples were spread on solid synthetic medium with 2% of L-arabinose and incubated at 30° C. for several days. Separate colonies were re-streaked twice on solid synthetic medium with L-arabinose. Finally, a shake flask containing synthetic medium with 2% of L-arabinose was inoculated with a single colony, and incubated for 4 days at 30° C. This culture was designated as strain IMS0003 (CBS 121879 deposited at the CBS on 20/09/07). Culture samples were taken, 30% of glycerol was added and samples were stored at −80° C.

Characterization of Strain IMS0003

[0182] Growth and product formation of strain IMS0003 was determined during an anaerobic batch fermentation on synthetic medium with a mixture of 30 g l^{-1} glucose, 15 g l^{-1} D-xylose and 15 g l^{-1} L-arabinose. The pre-culture for this anaerobic batch fermentation was prepared in a shake flasks containing 100 ml of synthetic medium with 2% L-arabinose, by inoculating with a −80° C. frozen stock of strain IMS0003, and incubated for 48 hours at 30° C.

[0183] In FIG. **7**, the fermentation profile of a mixture of 30 g l^{-1} glucose, 15 g l^{-1} D-xylose, and 15 g l^{-1} L-arabinose by strain IMS0003 is shown. Arabinose consumption started after glucose depletion. Within 70 hours, the glucose, xylose and arabinose were completely consumed. Xylose and arabinose were consumed simultaneously. At least 406 mM of ethanol was produced from the total of sugars, not taking into account the evaporation of the ethanol. The final ethanol concentration calculated from the cumulative CO_2 production was 572 mmol l^{-1}, corresponding to an ethanol yield of 0.46 g g^{-1} of total sugar. In contrast to the fermentation of a mixture of glucose, xylose and arabinose by strain IMS0002 (FIG. **5**C) or a mixed culture of strains IMS0002 and RWB218 (FIG. **5**D), strain IMS0003 did not produce detectable amounts of xylitol.

TABLE 1

S. cerevisiae strains used.

Strain	Characteristics	Reference
RWB217	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl pUGP$_{TPI}$-RPEl KanloxP-P_{TPI}::(−?, −1)RKIl {p415ADHXKS, PAKX002}	Kuyper et al. 2005a
RWB218	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl pUGP$_{TPI}$-RPEl KanloxP-P_{TPI}::(−?, −1)RKIl {p415ADHXKS1, pAKX002}	Kuyper et al. 2005b
RWB219	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl pUGP$_{TPI}$-RPEl KanloxP-P_{TPI}::(−?, −1)RKIl {p415ADHXKS1, pAKX002}	This work
RWB220	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl $_{P}$UGP$_{TPI}$-RPEl loxP-P_{TPI}::(−?, −1)RKIl	This work
IMS0001	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl pUGP$_{TPI}$-RPEl loxP-P_{TPI}::(−?, −1)RKIl {pRW231, PRW243}	This work
IMS0002	MATA ura3-52 leu2-112 loxP-P_{TPI}::(−266, −1)TALl gre3::hphMX pUGP$_{TPI}$-TKLl pUGP$_{TPI}$-RPEl loxP-P_{TPI}::(−?, −1)RKIl {pRW231, PRW243} selected for anaerobic growth on L-arabinose	This work

TABLE 2

Plasmids used

plasmid	characteristics	Reference
pRS305	Integration, LEU2	Gietz and Sugino, 1988
pAKX002	2μ, URA3, P_{TPI}-Piromyces xylA	Kuyper et al. 2003
p415ADHXKS1	CEN, LEU2, P_{ADH}-S.cerXKS1	Kuyper et al., 2005a
pRW229	integration, LEU2, P_{ADH}-S.cerXKS1	This work
pRW230	pAKX002 with P_{TDH3}-AraA	This work
pRW231	pAKX002 with P_{TDH3}-AraA and P_{HXT7}-AraD	This work
pRW243	LEU2, integration, P_{ADH}-ScXKS1-T_{CYC}, P_{PGI}-L.plantarumAraB-T_{ADHI}	This work

TABLE 3

oligos used in this work

Oligo	DNA sequence
AraA expression cassette	
SpeI5'Ptdh3 SEQ ID NO: 31	5'GACTAGTCGAGTTTATCATTATCAATACTGC3'
5'AraAPtdh SEQ ID NO: 32	5'CTCATAATCAGGTACTGATAACATTTTGTTTGTTTATGTGTGTTTATTC3'
Ptdh5'AraA SEQ ID NO: 33	5'GAATAAACACACATAAACAAACAAAATGTTATCAGTACCTGATTATGAG3
Tadh3'AraA SEQ ID NO: 34	5'AATCATAAATCATAAGAAATTCGCTTACTTTAAGAATGCCTTAGTCAT3'
3'AraATadh 1 SEQ ID NO: 35	5'ATGACTAAGGCATTCTTAAAGTAAGCGAATTTCTTATGATTTATGATT3'
3'Tadh 1 SpeI SEQ ID NO: 36	5'CACTAGTCTCGAGTGTGGAAGAACGATTACAACAGG3'
AraB expression cassette	
SacI5'Ppgi1 SEQ ID NO: 37	5'CGAGCTCGTGGGTGTATTGGATTATAGGAAG3'
5'AraBPpgi1 SEQ ID NO: 38	5'TTGGGCTGTTTCAACTAAATTCATTTTTAGGCTGGTATCTTGATTCTA3'
Ppgi5'AraB SEQ ID NO: 39	5'TAGAATCAAGATACCAGCCTAAAAATGAATTTAGTTGAAACAGCCCAA3'

TABLE 3-continued

oligos used in this work

Oligo	DNA sequence
Tadh3'AraB SEQ ID NO: 40	5'AATCATAAATCATAAGAAATTCGCTCTAATATTTGATTGCTTGCCCAG3'
3'AraBTadh 1 SEQ ID NO: 41	5'CTGGGCAAGCAATCAAATATTAGAGCGAATTTCTTATGATTTATGATT3'
3'Tadh 1 SacI SEQ ID NO: 42	5'TGAGCTCGTGTGGAAGAACGATTACAACAGG3'
AraD expression cassette	
SalI5'Phxt7 SEQ ID NO: 43	5'ACGCGTCGACTCGTAGGAACAATTTCGG3'
5'AraDPhxt SEQ ID NO: 44	5'CTTCTTGTTTTAATGCTTCTAGCATTTTTTGATTAAAATTAAAAAAACTT3'
Phxt5'AraD SEQ ID NO: 45	5'AAGTTTTTTTAATTTTAATCAAAAAATGCTAGAAGCATTAAAACAAGAAG3'
Tpgi3'AraD SEQ ID NO: 46	5'GGTATATATTTAAGAGCGATTTGTTTACTTGCGAACTGCATGATCC3'
3'AraDTpgi SEQ ID NO: 47	5'GGATCATGCAGTTCGCAAGTAAACAAATCGCTCTTAAATATATACC3'
3'TpgiSalI SEQ ID NO: 48	5'CGCAGTCGACCTTTTAAACAGTTGATGAGAACC3'

TABLE 4

Maximum observed specific glucose and arabinose consumption rates and ethanol production rates during anaerobic batch fermentations of *S. cerevisiae* IMS0002.

C-source	q_{glu} g h^{-1} g^{-1} DW	q_{ara} g h^{-1} g^{-1} DW	$q_{eth,\,glu}$ g h^{-1} g^{-1} DW	$q_{eth,\,ara}$ g h^{-1} g^{-1} DW
20 g l^{-1} arabinose	—	0.75 ± 0.04	—	0.31 ± 0.02
20 g l^{-1} glucose	2.08 ± 0.09	0.41 ± 0.01	0.69 ± 0.00	0.19 ± 0.00
20 g l^{-1} arabinose 30 g l^{-1} glucose	1.84 ± 0.04	0.23 ± 0.01	0.64 ± 0.03	0.08 ± 0.01
15 g l^{-1} xylose 15 g l^{-1} arabinose				

q_{glu}: specific glucose consumption rate
q_{ara}: specific arabinose consumption rate
$q_{eth,\,glu}$: specific ethanol production rate during growth on glucose
$q_{eth,\,ara}$: specific ethanol production rate during growth on arabinose

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 53

<210> SEQ ID NO 1
<211> LENGTH: 474
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 1

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Met Leu Ser Val Pro Asp Tyr Glu Phe Trp Phe Val Thr Gly Ser Gln
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His Leu Tyr Gly Glu Glu Gln Leu Lys Ser Val Ala Lys Asp Ala Gln
            20                  25                  30

Asp Ile Ala Asp Lys Leu Asn Ala Ser Gly Lys Leu Pro Tyr Lys Val
        35                  40                  45

Val Phe Lys Asp Val Met Thr Thr Ala Glu Ser Ile Thr Asn Phe Met
    50                  55                  60

Lys Glu Val Asn Tyr Asn Asp Lys Val Ala Gly Val Ile Thr Trp Met
65                  70                  75                  80

His Thr Phe Ser Pro Ala Lys Asn Trp Ile Arg Gly Thr Glu Leu Leu
                85                  90                  95

Gln Lys Pro Leu Leu His Leu Ala Thr Gln Tyr Leu Asn Asn Ile Pro
            100                 105                 110

Tyr Ala Asp Ile Asp Phe Asp Tyr Met Asn Leu Asn Gln Ser Ala His
        115                 120                 125

Gly Asp Arg Glu Tyr Ala Tyr Ile Asn Ala Arg Leu Gln Lys His Asn
    130                 135                 140

Lys Ile Val Tyr Gly Tyr Trp Gly Asp Glu Asp Val Gln Glu Gln Ile
145                 150                 155                 160

Ala Arg Trp Glu Asp Val Ala Val Ala Tyr Asn Glu Ser Phe Lys Val
                165                 170                 175

Lys Val Ala Arg Phe Gly Asp Thr Met Arg Asn Val Ala Val Thr Glu
            180                 185                 190

Gly Asp Lys Val Glu Ala Gln Ile Lys Met Gly Trp Thr Val Asp Tyr
        195                 200                 205

Tyr Gly Ile Gly Asp Leu Val Glu Glu Ile Asn Lys Val Ser Asp Ala
    210                 215                 220

Asp Val Asp Lys Glu Tyr Ala Asp Leu Glu Ser Arg Tyr Glu Met Val
225                 230                 235                 240
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Gln Val Asp Asn Asp Ala Asp Thr Tyr Lys His Ser Val Arg Val Gln
                245                 250                 255

Leu Ala Gln Tyr Leu Gly Ile Lys Arg Phe Leu Glu Arg Gly Gly Tyr
            260                 265                 270

Thr Ala Phe Thr Thr Asn Phe Glu Asp Leu Trp Gly Met Glu Gln Leu
        275                 280                 285

Pro Gly Leu Ala Ser Gln Leu Leu Ile Arg Asp Gly Tyr Gly Phe Gly
    290                 295                 300

Ala Glu Gly Asp Trp Lys Thr Ala Ala Leu Gly Arg Val Met Lys Ile
305                 310                 315                 320

Met Ser His Asn Lys Gln Thr Ala Phe Met Glu Asp Tyr Thr Leu Asp
                325                 330                 335

Leu Arg His Gly His Glu Ala Ile Leu Gly Ser His Met Leu Glu Val
            340                 345                 350

Asp Pro Ser Ile Ala Ser Asp Lys Pro Arg Val Glu Val His Pro Leu
        355                 360                 365

Asp Ile Gly Gly Lys Asp Asp Pro Ala Arg Leu Val Phe Thr Gly Ser
    370                 375                 380

Glu Gly Glu Ala Ile Asp Val Thr Val Ala Asp Phe Arg Asp Gly Phe
385                 390                 395                 400

Lys Met Ile Ser Tyr Ala Val Asp Ala Asn Lys Pro Glu Ala Glu Thr
                405                 410                 415

Pro Asn Leu Pro Val Ala Lys Gln Leu Trp Thr Pro Lys Met Gly Leu
            420                 425                 430

Lys Lys Gly Ala Leu Glu Trp Met Gln Ala Gly Gly Gly His His Thr
        435                 440                 445

Met Leu Ser Phe Ser Leu Thr Glu Glu Gln Met Glu Asp Tyr Ala Thr
    450                 455                 460

Met Val Gly Met Thr Lys Ala Phe Leu Lys
465                 470


<210> SEQ ID NO 2
<211> LENGTH: 1425
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 2

atgttatcag tacctgatta tgagttttgg tttgttaccg gttcacaaca cctttatggt     60

gaagaacaat tgaagtctgt tgctaaggat gcgcaagata ttgcggataa attgaatgca    120

agcggcaagt taccttataa agtagtcttt aaggatgtta tgacgacggc tgaaagtatc    180

accaacttta tgaaagaagt taattacaat gataaggtag ccggtgttat tacttggatg    240

cacacattct caccagctaa gaactggatt cgtggaactg aactgttaca aaaaccatta    300

ttacacttag caacgcaata tttgaataat attccatatg cagacattga ctttgattac    360

atgaacctta accaaagtgc ccatggcgac cgcgagtatg cctacattaa cgcccggttg    420

cagaaacata ataagattgt ttacggctat tggggcgatg aagatgtgca agagcagatt    480

gcacgttggg aagacgtcgc cgtagcgtac aatgagagct ttaaagttaa ggttgctcgc    540

tttggcgaca caatgcgtaa tgtggccgtt actgaaggtg acaaggttga agctcaaatt    600

aagatgggct ggacagttga ctattatggt atcggtgact tagttgaaga gatcaataag    660

gtttcggatg ctgatgttga taaggaatac gctgacttgg agtctcggta tgaaatggtc    720
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caagttgata acgatgcgga cacgtataaa cattcagttc gggttcaatt ggcacaatat 780

ctgggtatta agcggttctt agaaagaggc ggttacacag cctttaccac gaactttgaa 840

gatctttggg ggatggagca attacctggt ctagcttcac aattattaat tcgtgatggg 900

tatggttttg gtgctgaagg tgactggaag acggctgctt taggacgggt tatgaagatt 960

atgtctcaca acaagcaaac cgcctttatg gaagactaca cgttagactt gcgtcatggt 1020

catgaagcga tcttaggttc acacatgttg gaagttgatc cgtctatcgc aagtgataaa 1080

ccacgggtcg aagttcatcc attggatatt gggggtaaag atgatcctgc tcgcctagta 1140

tttactggtt cagaaggtga agcaattgat gtcaccgttg ccgatttccg tgatgggttc 1200

aagatgatta gctacgcggt agatgcgaat aagccagaag ccgaaacacc taatttacca 1260

gttgctaagc aattatggac cccaaagatg ggcttgaaga agggtgcact agaatggatg 1320

caagctggtg gtggtcacca cacgatgctg tccttctcgt taactgaaga acaaatggaa 1380

gactatgcaa ccatggttgg catgactaag gcattcttaa agtaa 1425

<210> SEQ ID NO 3
<211> LENGTH: 533
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 3

Met Asn Leu Val Glu Thr Ala Gln Ala Ile Lys Thr Gly Lys Val Ser
1 5 10 15

Leu Gly Ile Glu Leu Gly Ser Thr Arg Ile Lys Ala Val Leu Ile Thr
20 25 30

Asp Asp Phe Asn Thr Ile Ala Ser Gly Ser Tyr Val Trp Glu Asn Gln
35 40 45

Phe Val Asp Gly Thr Trp Thr Tyr Ala Leu Glu Asp Val Trp Thr Gly
50 55 60

Ile Gln Gln Ser Tyr Thr Gln Leu Ala Ala Asp Val Arg Ser Lys Tyr
65 70 75 80

His Met Ser Leu Lys His Ile Asn Ala Ile Gly Ile Ser Ala Met Met
85 90 95

His Gly Tyr Leu Ala Phe Asp Gln Gln Ala Lys Leu Leu Val Pro Phe
100 105 110

Arg Thr Trp Arg Asn Asn Ile Thr Gly Gln Ala Ala Asp Glu Leu Thr
115 120 125

Glu Leu Phe Asp Phe Asn Ile Pro Gln Arg Trp Ser Ile Ala His Leu
130 135 140

Tyr Gln Ala Ile Leu Asn Asn Glu Ala His Val Lys Gln Val Asp Phe
145 150 155 160

Ile Thr Thr Leu Ala Gly Tyr Val Thr Trp Lys Leu Ser Gly Glu Lys
165 170 175

Val Leu Gly Ile Gly Asp Ala Ser Gly Val Phe Pro Ile Asp Glu Thr
180 185 190

Thr Asp Thr Tyr Asn Gln Thr Met Leu Thr Lys Phe Ser Gln Leu Asp
195 200 205

Lys Val Lys Pro Tyr Ser Trp Asp Ile Arg His Ile Leu Pro Arg Val
210 215 220

Leu Pro Ala Gly Ala Ile Ala Gly Lys Leu Thr Ala Ala Gly Ala Ser

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225                 230                 235                 240

Leu Leu Asp Gln Ser Gly Thr Leu Asp Ala Gly Ser Val Ile Ala Pro
                245                 250                 255

Pro Glu Gly Asp Ala Gly Thr Gly Met Val Gly Thr Asn Ser Val Arg
            260                 265                 270

Lys Arg Thr Gly Asn Ile Ser Val Gly Thr Ser Ala Phe Ser Met Asn
        275                 280                 285

Val Leu Asp Lys Pro Leu Ser Lys Val Tyr Arg Asp Ile Asp Ile Val
    290                 295                 300

Met Thr Pro Asp Gly Ser Pro Val Ala Met Val His Val Asn Asn Cys
305                 310                 315                 320

Ser Ser Asp Ile Asn Ala Trp Ala Thr Ile Phe Arg Glu Phe Ala Ala
                325                 330                 335

Arg Leu Gly Met Glu Leu Lys Pro Asp Arg Leu Tyr Glu Thr Leu Phe
            340                 345                 350

Leu Glu Ser Thr Arg Ala Asp Ala Asp Ala Gly Gly Leu Ala Asn Tyr
        355                 360                 365

Ser Tyr Gln Ser Gly Glu Asn Ile Thr Lys Ile Gln Ala Gly Arg Pro
    370                 375                 380

Leu Phe Val Arg Thr Pro Asn Ser Lys Phe Ser Leu Pro Asn Phe Met
385                 390                 395                 400

Leu Thr Gln Leu Tyr Ala Ala Phe Ala Pro Leu Gln Leu Gly Met Asp
                405                 410                 415

Ile Leu Val Asn Glu Glu His Val Gln Thr Asp Val Met Ile Ala Gln
            420                 425                 430

Gly Gly Leu Phe Arg Thr Pro Val Ile Gly Gln Gln Val Leu Ala Asn
        435                 440                 445

Ala Leu Asn Ile Pro Ile Thr Val Met Ser Thr Ala Gly Glu Gly Gly
    450                 455                 460

Pro Trp Gly Met Ala Val Leu Ala Asn Phe Ala Cys Arg Gln Thr Ala
465                 470                 475                 480

Met Asn Leu Glu Asp Phe Leu Asp Gln Glu Val Phe Lys Glu Pro Glu
                485                 490                 495

Ser Met Thr Leu Ser Pro Glu Pro Glu Arg Val Ala Gly Tyr Arg Glu
            500                 505                 510

Phe Ile Gln Arg Tyr Gln Ala Gly Leu Pro Val Glu Ala Ala Ala Gly
        515                 520                 525

Gln Ala Ile Lys Tyr
    530


<210> SEQ ID NO 4
<211> LENGTH: 1602
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 4

atgaatttag ttgaaacagc ccaagcgatt aaaactggca aagtttcttt aggaattgag     60

cttggctcaa ctcgaattaa agccgttttg atcacggacg attttaatac gattgcttcg    120

ggaagttacg tttgggaaaa ccaatttgtt gatggtactt ggacttacgc acttgaagat    180

gtctggaccg gaattcaaca aagttatacg caattagcag cagatgtccg cagtaaatat    240

cacatgagtt tgaagcatat caatgctatt ggcattagtg ccatgatgca cggataccta    300
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gcatttgatc aacaagcgaa attattagtt ccgtttcgga cttggcgtaa taacattacg 360

gggcaagcag cagatgaatt gaccgaatta tttgatttca acattccaca acggtggagt 420

atcgcgcact tataccaggc aatcttaaat aatgaagcgc acgttaaaca ggtggacttc 480

ataacaacgc tggctggcta tgtaacctgg aaattgtcgg gtgagaaagt tctaggaatc 540

ggtgatgcgt ctggcgtttt cccaattgat gaaacgactg acacatacaa tcagacgatg 600

ttaaccaagt ttagccaact tgacaaagtt aaaccgtatt catgggatat ccggcatatt 660

ttaccgcggg ttttaccagc gggagccatt gctggaaagt taacggctgc cggggcgagc 720

ttacttgatc agagcggcac gctcgacgct ggcagtgtta ttgcaccgcc agaaggggat 780

gctggaacag gaatggtcgg tacgaacagc gtccgtaaac gcacgggtaa catctcggtg 840

ggaacctcag cattttcgat gaacgttcta gataaaccat tgtctaaagt ctatcgcgat 900

attgatattg ttatgacgcc agatgggtca ccagttgcaa tggtgcatgt taataattgt 960

tcatcagata ttaatgcgtg ggcaacgatt tttcgtgagt ttgcagcccg gttgggaatg 1020

gaattgaaac cggatcgatt atatgaaacg ttattcttgg aatcaactcg cgctgatgcg 1080

gatgctggag ggttggctaa ttatagttat caatccggtg agaatattac taagattcaa 1140

gctggtcggc cgctatttgt acggacacca aacagtaaat ttagtttacc gaactttatg 1200

ttgacccaat tatatgcggc gttcgcaccc ctccaacttg gtatggatat tcttgttaac 1260

gaagaacatg ttcaaacgga cgttatgatt gcacagggtg gattgttccg aacgccggta 1320

attggccaac aagtattggc caacgcactg aacattccga ttactgtaat gagtactgct 1380

ggtgaaggcg gcccatgggg gatggcagtg ttagccaact ttgcttgtcg gcaaactgca 1440

atgaacctag aagatttctt agatcaagaa gtctttaaag agccagaaag tatgacgttg 1500

agtccagaac cggaacgggt ggccggatat cgtgaattta ttcaacgtta tcaagctggc 1560

ttaccagttg aagcagcggc tgggcaagca atcaaatatt ag 1602

<210> SEQ ID NO 5
<211> LENGTH: 242
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 5

Met Leu Glu Ala Leu Lys Gln Glu Val Tyr Glu Ala Asn Met Gln Leu
1 5 10 15

Pro Lys Leu Gly Leu Val Thr Phe Thr Trp Gly Asn Val Ser Gly Ile
20 25 30

Asp Arg Glu Lys Gly Leu Phe Val Ile Lys Pro Ser Gly Val Asp Tyr
35 40 45

Gly Glu Leu Lys Pro Ser Asp Leu Val Val Val Asn Leu Gln Gly Glu
50 55 60

Val Val Glu Gly Lys Leu Asn Pro Ser Ser Asp Thr Pro Thr His Thr
65 70 75 80

Val Leu Tyr Asn Ala Phe Pro Asn Ile Gly Gly Ile Val His Thr His
85 90 95

Ser Pro Trp Ala Val Ala Tyr Ala Ala Ala Gln Met Asp Val Pro Ala
100 105 110

Met Asn Thr Thr His Ala Asp Thr Phe Tyr Gly Asp Val Pro Ala Ala
115 120 125

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Asp Ala Leu Thr Lys Glu Glu Ile Glu Ala Asp Tyr Glu Gly Asn Thr
130 135 140

Gly Lys Thr Ile Val Lys Thr Phe Gln Glu Arg Gly Leu Asp Tyr Glu
145 150 155 160

Ala Val Pro Ala Ser Leu Val Ser Gln His Gly Pro Phe Ala Trp Gly
165 170 175

Pro Thr Pro Ala Lys Ala Val Tyr Asn Ala Lys Val Leu Glu Val Val
180 185 190

Ala Glu Glu Asp Tyr His Thr Ala Gln Leu Thr Arg Ala Ser Ser Glu
195 200 205

Leu Pro Gln Tyr Leu Leu Asp Lys His Tyr Leu Arg Lys His Gly Ala
210 215 220

Ser Ala Tyr Tyr Gly Gln Asn Asn Ala His Ser Lys Asp His Ala Val
225 230 235 240

Arg Lys

<210> SEQ ID NO 6
<211> LENGTH: 729
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 6

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ctggttactt ttacctgggg caatgtctcg ggcattgacc gggaaaaagg cctattcgtg 120

atcaagccat ctggtgttga ttatggtgaa ttaaaaccaa gcgatttagt cgttgttaac 180

ttacagggtg aagtggttga aggtaaacta aatccgtcta gtgatacgcc gactcatacg 240

gtgttatata acgcttttcc taatattggc ggaattgtcc atactcattc gccatgggca 300

gttgcctatg cagctgctca aatggatgtg ccagctatga acacgaccca tgctgatacg 360

ttctatggtg acgtgccggc cgcggatgcg ctgactaagg aagaaattga agcagattat 420

gaaggcaaca cgggtaaaac cattgtgaag acgttccaag aacggggcct cgattatgaa 480

gctgtaccag cctcattagt cagccagcac ggcccatttg cttggggacc aacgccagct 540

aaagccgttt acaatgctaa agtgttggaa gtggttgccg aagaagatta tcatactgcg 600

caattgaccc gtgcaagtag cgaattacca caatatttat tagataagca ttatttacgt 660

aagcatggtg caagtgccta ttatggtcaa aataatgcgc attctaagga tcatgcagtt 720

cgcaagtaa 729

<210> SEQ ID NO 7
<211> LENGTH: 437
<212> TYPE: PRT
<213> ORGANISM: Piromyces sp.

<400> SEQUENCE: 7

Met Ala Lys Glu Tyr Phe Pro Gln Ile Gln Lys Ile Lys Phe Glu Gly
1 5 10 15

Lys Asp Ser Lys Asn Pro Leu Ala Phe His Tyr Tyr Asp Ala Glu Lys
20 25 30

Glu Val Met Gly Lys Lys Met Lys Asp Trp Leu Arg Phe Ala Met Ala
35 40 45

Trp Trp His Thr Leu Cys Ala Glu Gly Ala Asp Gln Phe Gly Gly Gly

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    50                  55                  60

Thr Lys Ser Phe Pro Trp Asn Glu Gly Thr Asp Ala Ile Glu Ile Ala
65                  70                  75                  80

Lys Gln Lys Val Asp Ala Gly Phe Glu Ile Met Gln Lys Leu Gly Ile
                85                  90                  95

Pro Tyr Tyr Cys Phe His Asp Val Asp Leu Val Ser Glu Gly Asn Ser
            100                 105                 110

Ile Glu Glu Tyr Glu Ser Asn Leu Lys Ala Val Val Ala Tyr Leu Lys
        115                 120                 125

Glu Lys Gln Lys Glu Thr Gly Ile Lys Leu Leu Trp Ser Thr Ala Asn
    130                 135                 140

Val Phe Gly His Lys Arg Tyr Met Asn Gly Ala Ser Thr Asn Pro Asp
145                 150                 155                 160

Phe Asp Val Val Ala Arg Ala Ile Val Gln Ile Lys Asn Ala Ile Asp
                165                 170                 175

Ala Gly Ile Glu Leu Gly Ala Glu Asn Tyr Val Phe Trp Gly Gly Arg
            180                 185                 190

Glu Gly Tyr Met Ser Leu Leu Asn Thr Asp Gln Lys Arg Glu Lys Glu
        195                 200                 205

His Met Ala Thr Met Leu Thr Met Ala Arg Asp Tyr Ala Arg Ser Lys
    210                 215                 220

Gly Phe Lys Gly Thr Phe Leu Ile Glu Pro Lys Pro Met Glu Pro Thr
225                 230                 235                 240

Lys His Gln Tyr Asp Val Asp Thr Glu Thr Ala Ile Gly Phe Leu Lys
                245                 250                 255

Ala His Asn Leu Asp Lys Asp Phe Lys Val Asn Ile Glu Val Asn His
            260                 265                 270

Ala Thr Leu Ala Gly His Thr Phe Glu His Glu Leu Ala Cys Ala Val
        275                 280                 285

Asp Ala Gly Met Leu Gly Ser Ile Asp Ala Asn Arg Gly Asp Tyr Gln
    290                 295                 300

Asn Gly Trp Asp Thr Asp Gln Phe Pro Ile Asp Gln Tyr Glu Leu Val
305                 310                 315                 320

Gln Ala Trp Met Glu Ile Ile Arg Gly Gly Gly Phe Val Thr Gly Gly
                325                 330                 335

Thr Asn Phe Asp Ala Lys Thr Arg Arg Asn Ser Thr Asp Leu Glu Asp
            340                 345                 350

Ile Ile Ile Ala His Val Ser Gly Met Asp Ala Met Ala Arg Ala Leu
        355                 360                 365

Glu Asn Ala Ala Lys Leu Leu Gln Glu Ser Pro Tyr Thr Lys Met Lys
    370                 375                 380

Lys Glu Arg Tyr Ala Ser Phe Asp Ser Gly Ile Gly Lys Asp Phe Glu
385                 390                 395                 400

Asp Gly Lys Leu Thr Leu Glu Gln Val Tyr Glu Tyr Gly Lys Lys Asn
                405                 410                 415

Gly Glu Pro Lys Gln Thr Ser Gly Lys Gln Glu Leu Tyr Glu Ala Ile
            420                 425                 430

Val Ala Met Tyr Gln
        435


<210> SEQ ID NO 8
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<211> LENGTH: 1669
<212> TYPE: DNA
<213> ORGANISM: Piromyces sp.

<400> SEQUENCE: 8

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taagaatcca ttagccttcc actactacga tgctgaaaag gaagtcatgg gtaagaaaat 120

gaaggattgg ttacgtttcg ccatggcctg gtggcacact ctttgcgccg aaggtgctga 180

ccaattcggt ggaggtacaa agtctttccc atggaacgaa ggtactgatg ctattgaaat 240

tgccaagcaa aaggttgatg ctggtttcga aatcatgcaa aagcttggta ttccatacta 300

ctgtttccac gatgttgatc ttgtttccga aggtaactct attgaagaat acgaatccaa 360

ccttaaggct gtcgttgctt acctcaagga aaagcaaaag gaaaccggta ttaagcttct 420

ctggagtact gctaacgtct tcggtcacaa gcgttacatg aacggtgcct ccactaaccc 480

agactttgat gttgtcgccc gtgctattgt tcaaattaag aacgccatag acgccggtat 540

tgaacttggt gctgaaaact acgtcttctg gggtggtcgt gaaggttaca tgagtctcct 600

taacactgac caaaagcgtg aaaaggaaca catggccact atgcttacca tggctcgtga 660

ctacgctcgt tccaagggat tcaagggtac tttcctcatt gaaccaaagc caatggaacc 720

aaccaagcac caatacgatg ttgacactga aaccgctatt ggtttcctta aggcccacaa 780

cttagacaag gacttcaagg tcaacattga agttaaccac gctactcttg ctggtcacac 840

tttcgaacac gaacttgcct gtgctgttga tgctggtatg ctcggttcca ttgatgctaa 900

ccgtggtgac taccaaaacg gttgggatac tgatcaattc ccaattgatc aatacgaact 960

cgtccaagct tggatggaaa tcatccgtgg tggtggtttc gttactggtg gtaccaactt 1020

cgatgccaag actcgtcgta actctactga cctcgaagac atcatcattg cccacgtttc 1080

tggtatggat gctatggctc gtgctcttga aaacgctgcc aagctcctcc aagaatctcc 1140

atacaccaag atgaagaagg aacgttacgc ttccttcgac agtggtattg gtaaggactt 1200

tgaagatggt aagctcaccc tcgaacaagt ttacgaatac ggtaagaaga acggtgaacc 1260

aaagcaaact tctggtaagc aagaactcta cgaagctatt gttgccatgt accaataagt 1320

taatcgtagt taaattggta aaataattgt aaaatcaata aacttgtcaa tcctccaatc 1380

aagtttaaaa gatcctatct ctgtactaat taaatatagt acaaaaaaaa atgtataaac 1440

aaaaaaaagt ctaaaagacg gaagaattta atttagggaa aaaataaaaa taataataaa 1500

caatagataa atcctttata ttaggaaaat gtcccattgt attattttca tttctactaa 1560

aaaagaaagt aaataaaaca caagaggaaa ttttcccttt ttttttttt tgtaataaat 1620

tttatgcaaa tataaatata aataaaataa taaaaaaaaa aaaaaaaaa 1669

<210> SEQ ID NO 9
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 9

Met Leu Gln Thr Lys Asp Tyr Glu Phe Trp Phe Val Thr Gly Ser Gln
1 5 10 15

His Leu Tyr Gly Glu Glu Thr Leu Glu Leu Val Asp Gln His Ala Lys
20 25 30

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Ser Ile Cys Glu Gly Leu Ser Gly Ile Ser Ser Arg Tyr Lys Ile Thr
        35                  40                  45

His Lys Pro Val Val Thr Ser Pro Glu Thr Ile Arg Glu Leu Leu Arg
    50                  55                  60

Glu Ala Glu Tyr Ser Glu Thr Cys Ala Gly Ile Ile Thr Trp Met His
65                  70                  75                  80

Thr Phe Ser Pro Ala Lys Met Trp Ile Glu Gly Leu Ser Ser Tyr Gln
                85                  90                  95

Lys Pro Leu Met His Leu His Thr Gln Tyr Asn Arg Asp Ile Pro Trp
            100                 105                 110

Gly Thr Ile Asp Met Asp Phe Met Asn Ser Asn Gln Ser Ala His Gly
        115                 120                 125

Asp Arg Glu Tyr Gly Tyr Ile Asn Ser Arg Met Gly Leu Ser Arg Lys
    130                 135                 140

Val Ile Ala Gly Tyr Trp Asp Asp Glu Glu Val Lys Lys Glu Met Ser
145                 150                 155                 160

Gln Trp Met Asp Thr Ala Ala Ala Leu Asn Glu Ser Arg His Ile Lys
                165                 170                 175

Val Ala Arg Phe Gly Asp Asn Met Arg His Val Ala Val Thr Asp Gly
            180                 185                 190

Asp Lys Val Gly Ala His Ile Gln Phe Gly Trp Gln Val Asp Gly Tyr
        195                 200                 205

Gly Ile Gly Asp Leu Val Glu Val Met Asp Arg Ile Thr Asp Asp Glu
    210                 215                 220

Val Asp Thr Leu Tyr Ala Glu Tyr Asp Arg Leu Tyr Val Ile Ser Glu
225                 230                 235                 240

Glu Thr Lys Arg Asp Glu Ala Lys Val Ala Ser Ile Lys Glu Gln Ala
                245                 250                 255

Lys Ile Glu Leu Gly Leu Thr Ala Phe Leu Glu Gln Gly Gly Tyr Thr
            260                 265                 270

Ala Phe Thr Thr Ser Phe Glu Val Leu His Gly Met Lys Gln Leu Pro
        275                 280                 285

Gly Leu Ala Val Gln Arg Leu Met Glu Lys Gly Tyr Gly Phe Ala Gly
    290                 295                 300

Glu Gly Asp Trp Lys Thr Ala Ala Leu Val Arg Met Met Lys Ile Met
305                 310                 315                 320

Ala Lys Gly Lys Arg Thr Ser Phe Met Glu Asp Tyr Thr Tyr His Phe
                325                 330                 335

Glu Pro Gly Asn Glu Met Ile Leu Gly Ser His Met Leu Glu Val Cys
            340                 345                 350

Pro Thr Val Ala Leu Asp Gln Pro Lys Ile Glu Val His Ser Leu Ser
        355                 360                 365

Ile Gly Gly Lys Glu Asp Pro Ala Arg Leu Val Phe Asn Gly Ile Ser
    370                 375                 380

Gly Ser Ala Ile Gln Ala Ser Ile Val Asp Ile Gly Gly Arg Phe Arg
385                 390                 395                 400

Leu Val Leu Asn Glu Val Asn Gly Gln Glu Ile Glu Lys Asp Met Pro
                405                 410                 415

Asn Leu Pro Val Ala Arg Val Leu Trp Lys Pro Glu Pro Ser Leu Lys
            420                 425                 430

Thr Ala Ala Glu Ala Trp Ile Leu Ala Gly Gly Ala His His Thr Cys
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        435                 440                 445

Leu Ser Tyr Glu Leu Thr Ala Glu Gln Met Leu Asp Trp Ala Glu Met
    450                 455                 460

Ala Gly Ile Glu Ser Val Leu Ile Ser Arg Asp Thr Thr Ile His Lys
465                 470                 475                 480

Leu Lys His Glu Leu Lys Trp Asn Glu Ala Leu Tyr Arg Leu Gln Lys
                485                 490                 495


<210> SEQ ID NO 10
<211> LENGTH: 1511
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 10

atgagaaagg ggcagtttac atgcttcaga caaaggatta tgaattctgg tttgtgacag     60

gaagccagca cctatacggg gaagagacgc tggaactcgt agatcagcat gctaaaagca    120

tttgtgaggg gctcagcggg atttcttcca gatataaaat cactcataag cccgtcgtca    180

cttcaccgga aaccattaga gagctgttaa gagaagcgga gtacagtgag acatgtgctg    240

gcatcattac atggatgcac acattttccc ctgcaaaaat gtggatagaa ggcctttcct    300

cttatcaaaa accgcttatg catttgcata cccaatataa tcgcgatatc ccgtggggta    360

cgattgacat ggattttatg aacagcaacc aatccgcgca tggcgatcga gagtacggtt    420

acatcaactc gagaatgggg cttagccgaa aagtcattgc cggctattgg gatgatgaag    480

aagtgaaaaa agaaatgtcc cagtggatgg atacggcggc tgcattaaat gaaagcagac    540

atattaaggt tgccagattt ggagataaca tgcgtcatgt cgcggtaacg gacggagaca    600

aggtgggagc gcatattcaa tttggctggc aggttgacgg atatggcatc ggggatctcg    660

ttgaagtgat ggatcgcatt acggacgacg aggttgacac gctttatgcc gagtatgaca    720

gactatatgt gatcagtgag gaaacaaaac gtgacgaagc aaaggtagcg tccattaaag    780

aacaggcgaa aattgaactt ggattaaccg cttttcttga gcaaggcgga tacacagcgt    840

ttacgacatc gtttgaagtg ctgcacggaa tgaaacagct gccgggactt gccgttcagc    900

gcctgatgga gaaaggctat gggtttgccg gtgaaggaga ttggaagaca gcggcccttg    960

tacggatgat gaaaatcatg gctaaaggaa aaagaacttc cttcatggaa gattacacgt   1020

accattttga accgggaaat gaaatgattc tgggctctca catgcttgaa gtgtgtccga   1080

ctgtcgcttt ggatcagccg aaaatcgagg ttcattcgct ttcgattggc ggcaaagagg   1140

accctgcgcg tttggtattt aacggcatca gcggttctgc cattcaagct agcattgttg   1200

atattggcgg gcgtttccgc cttgtgctga atgaagtcaa cggccaggaa attgaaaaag   1260

acatgccgaa tttaccggtt gcccgtgttc tctggaagcc ggagccgtca ttgaaaacag   1320

cagcggaggc atggatttta gccggcggtg cacaccatac ctgcctgtct tatgaactga   1380

cagcggagca aatgcttgat tgggcggaaa tggcgggaat cgaaagtgtt ctcatttccc   1440

gtgatacgac aattcataaa ctgaaacacg agttaaaatg gaacgaggcg ctttaccggc   1500

ttcaaaagta g                                                        1511


<210> SEQ ID NO 11
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: E. coli
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-continued

<400> SEQUENCE: 11

```
Met Ala Ile Ala Ile Gly Leu Asp Phe Gly Ser Asp Ser Val Arg Ala
1               5                   10                  15

Leu Ala Val Asp Cys Ala Ser Gly Glu Glu Ile Ala Thr Ser Val Glu
            20                  25                  30

Trp Tyr Pro Arg Trp Gln Lys Gly Gln Phe Cys Asp Ala Pro Asn Asn
        35                  40                  45

Gln Phe Arg His His Pro Arg Asp Tyr Ile Glu Ser Met Glu Ala Ala
    50                  55                  60

Leu Lys Thr Val Leu Ala Glu Leu Ser Val Glu Gln Arg Ala Ala Val
65                  70                  75                  80

Val Gly Ile Gly Val Asp Ser Thr Gly Ser Thr Pro Ala Pro Ile Asp
                85                  90                  95

Ala Asp Gly Asn Val Leu Ala Leu Arg Pro Glu Phe Ala Glu Asn Pro
            100                 105                 110

Asn Ala Met Phe Val Leu Trp Lys Asp His Thr Ala Val Glu Arg Ser
        115                 120                 125

Glu Glu Ile Thr Arg Leu Cys His Ala Pro Gly Asn Val Asp Tyr Ser
    130                 135                 140

Arg Tyr Ile Gly Gly Ile Tyr Ser Ser Glu Trp Phe Trp Ala Lys Ile
145                 150                 155                 160

Leu His Val Thr Arg Gln Asp Ser Ala Val Ala Gln Ser Ala Ala Ser
                165                 170                 175

Trp Ile Glu Leu Cys Asp Trp Val Pro Ala Leu Leu Ser Gly Thr Thr
            180                 185                 190

Arg Pro Gln Asp Ile Arg Arg Gly Arg Cys Ser Ala Gly His Lys Ser
        195                 200                 205

Leu Trp His Glu Ser Trp Gly Gly Leu Pro Pro Ala Ser Phe Phe Asp
    210                 215                 220

Glu Leu Asp Pro Ile Leu Asn Arg His Leu Pro Ser Pro Leu Phe Thr
225                 230                 235                 240

Asp Thr Trp Thr Ala Asp Ile Pro Val Gly Thr Leu Cys Pro Glu Trp
                245                 250                 255

Ala Gln Arg Leu Gly Leu Pro Glu Ser Val Val Ile Ser Gly Gly Ala
            260                 265                 270

Phe Asp Cys His Met Gly Ala Val Gly Ala Gly Ala Gln Pro Asn Ala
        275                 280                 285

Leu Val Lys Val Ile Gly Thr Ser Thr Cys Asp Ile Leu Ile Ala Asp
    290                 295                 300

Lys Gln Ser Val Gly Glu Arg Ala Val Lys Gly Ile Cys Gly Gln Val
305                 310                 315                 320

Asp Gly Ser Val Val Pro Gly Phe Ile Gly Leu Glu Ala Gly Gln Ser
                325                 330                 335

Ala Phe Gly Asp Ile Tyr Ala Trp Phe Gly Arg Val Leu Ser Trp Pro
            340                 345                 350

Leu Glu Gln Leu Ala Ala Gln His Pro Glu Leu Lys Ala Gln Ile Asn
        355                 360                 365

Ala Ser Gln Lys Gln Leu Leu Pro Ala Leu Thr Glu Ala Trp Ala Lys
    370                 375                 380

Asn Pro Ser Leu Asp His Leu Pro Val Val Leu Asp Trp Phe Asn Gly
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385                 390                 395                 400

Arg Arg Ser Pro Asn Ala Asn Gln Arg Leu Lys Gly Val Ile Thr Asp
                405                 410                 415

Leu Asn Leu Ala Thr Asp Ala Pro Leu Leu Phe Gly Gly Leu Ile Ala
            420                 425                 430

Ala Thr Ala Phe Gly Ala Arg Ala Ile Met Glu Cys Phe Thr Asp Gln
        435                 440                 445

Gly Ile Ala Val Asn Asn Val Met Ala Leu Gly Gly Ile Ala Arg Lys
    450                 455                 460

Asn Gln Val Ile Met Gln Ala Cys Cys Asp Val Leu Asn Arg Pro Leu
465                 470                 475                 480

Gln Ile Val Ala Ser Asp Gln Cys Cys Ala Leu Gly Ala Ala Ile Phe
                485                 490                 495

Ala Ala Val Ala Ala Lys Val His Ala Asp Ile Pro Ser Ala Gln Gln
            500                 505                 510

Lys Met Ala Ser Ala Val Glu Lys Thr Leu Gln Pro Arg Ser Glu Gln
        515                 520                 525

Ala Gln Arg Phe Glu Gln Leu Tyr Arg Arg Tyr Gln Gln Trp Ala Met
    530                 535                 540

Ser Ala Glu Gln His Tyr Leu Pro Thr Ser Ala Pro Ala Gln Ala Ala
545                 550                 555                 560

Gln Ala Val Ala Thr Leu
                565


<210> SEQ ID NO 12
<211> LENGTH: 1453
<212> TYPE: DNA
<213> ORGANISM: E. coli

<400> SEQUENCE: 12

atggcgattg caattggcct cgattttggc agtgattctg tgcgagcttt ggcggtggac     60

tgcgccagcg gtgaagagat cgccaccagc gtagagtggt atccccgttg gcaaaaaggg    120

caattttgtg atgccccgaa taaccagttc cgtcatcatc cgcgtgacta cattgagtca    180

atggaagcgg cactgaaaac cgtgcttgca gagcttagcg tcgaacagcg cgcagctgtg    240

gtcgggattg gcgttgacag taccggctcg acgcccgcac cgattgatgc cgacggtaac    300

gtgctggcgc tgcgcccgga gtttgccgaa aacccgaacg cgatgttcgt attgtggaaa    360

gaccacactg cggttgaaag aagcgaagag attacccgtt tgtgccacgc gccgggcaat    420

gttgactact cccgctatat tggcggtatt tattccagcg aatggttctg ggcaaaaatc    480

ctgcatgtga ctcgccagga cagcgccgtg gcgcaatctg ccgcatcgtg gattgagctg    540

tgcgactggg tgccagctct gctttccggt accacccgcc cgcaggatat tcgtcgcgga    600

cgttgcagcg ccgggcataa atctctgtgg cacgaaagct ggggcggctt gccgccagcc    660

agtttctttg atgagctgga cccgatcctc aatcgccatt tgccttcccc gctgttcact    720

gacacctgga ctgccgatat tccggtgggc accttatgcc cggaatgggc gcagcgtctc    780

ggcctgcctg aaagcgtggt gatttccggc ggcgcgtttg actgccatat gggcgcagtt    840

ggcgcaggcg cacagcctaa cgcactggta aaagttatcg gtacttccac ctgcgacatt    900

ctgattgccg acaaacagag cgttggcgag cgggcagtta aaggtatttg cggtcaggtt    960

gatggcagcg tggtgcctgg atttatcggt ctggaagcag gccaatcggc gtttggtgat   1020
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atctacgcct ggttcggtcg cgtactcagc tggccgctgg aacagcttgc cgcccagcat 1080

ccggaactga aagcgcaaat caacgccagc cagaaacaac tgcttccggc gctgaccgaa 1140

gcatgggcca aaaatccgtc tctggatcac ctgccggtgg tgctcgactg gtttaacggt 1200

cgtcgctcgc caaacgctaa ccaacgcctg aaaggggtga ttaccgatct taacctcgct 1260

accgacgctc cgctgctgtt cggcggtttg attgctgcca ccgcctttgg cgcacgcgca 1320

atcatggagt gctttaccga tcaggggatc gccgtcaata acgtgatggc gctgggcggc 1380

atcgcgcgga aaaaccaagt cattatgcag gcctgctgcg acgtgctgaa tcgcccgctg 1440

caaattgttg cct 1453

<210> SEQ ID NO 13
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: E. coli

<400> SEQUENCE: 13

Met Leu Glu Asp Leu Lys Arg Gln Val Leu Glu Ala Asn Leu Ala Leu
1 5 10 15

Pro Lys His Asn Leu Val Thr Leu Thr Trp Gly Asn Val Ser Ala Val
20 25 30

Asp Arg Glu Arg Gly Val Phe Val Ile Lys Pro Ser Gly Val Asp Tyr
35 40 45

Ser Ile Met Thr Ala Asp Asp Met Val Val Val Ser Ile Glu Thr Gly
50 55 60

Glu Val Val Glu Gly Ala Lys Lys Pro Ser Ser Asp Thr Pro Thr His
65 70 75 80

Arg Leu Leu Tyr Gln Ala Phe Pro Ser Ile Gly Gly Ile Val His Thr
85 90 95

His Ser Arg His Ala Thr Ile Trp Ala Gln Ala Gly Gln Ser Ile Pro
100 105 110

Ala Thr Gly Thr Thr His Ala Asp Tyr Phe Tyr Gly Thr Ile Pro Cys
115 120 125

Thr Arg Lys Met Thr Asp Ala Glu Ile Asn Gly Glu Tyr Glu Trp Glu
130 135 140

Thr Gly Asn Val Ile Val Glu Thr Phe Glu Lys Gln Gly Ile Asp Ala
145 150 155 160

Ala Gln Met Pro Gly Val Leu Val His Ser His Gly Pro Phe Ala Trp
165 170 175

Gly Lys Asn Ala Glu Asp Ala Val His Asn Ala Ile Val Leu Glu Glu
180 185 190

Val Ala Tyr Met Gly Ile Phe Cys Arg Gln Leu Ala Pro Gln Leu Pro
195 200 205

Asp Met Gln Gln Thr Leu Leu Asn Lys His Tyr Leu Arg Lys His Gly
210 215 220

Ala Lys Ala Tyr Tyr Gly Gln
225 230

<210> SEQ ID NO 14
<211> LENGTH: 696
<212> TYPE: DNA
<213> ORGANISM: E. coli

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<400> SEQUENCE: 14

atgttagaag atctcaaacg ccaggtatta gaggccaacc tggcgctgcc aaaacataac 60

ctggtcacgc tcacatgggg caacgtcagc gccgttgatc gcgagcgcgg cgtctttgtg 120

atcaaacctt ccggcgtcga ttacagcatc atgaccgctg acgatatggt cgtggttagc 180

atcgaaaccg gtgaagtggt tgaaggtgcg aaaaagccct cctccgatac gccaactcac 240

cgactgctct atcaggcatt cccgtccatt ggcggcattg tgcacacaca ctcgcgccac 300

gccactatct gggcgcaggc gggccagtcg attccagcaa ccggcaccac ccacgccgac 360

tatttctacg gcaccattcc ctgcacccgc aaaatgaccg acgcagaaat caacggtgaa 420

tatgagtggg aaaccggtaa cgtcatcgta gaaaccttcg aaaaacaggg tatcgatgca 480

gcgcaaatgc ccggcgtcct ggtccattct cacggcccat ttgcatgggg caaaaatgcc 540

gaagatgcgg tgcataacgc catcgtgctg gaagaggtcg cttatatggg gatattctgc 600

cgtcagttag cgccgcagtt accggatatg cagcaaacgc tgctgaataa acactatctg 660

cgtaagcatg gcgcgaaggc atattacggg cagtaa 696

<210> SEQ ID NO 15
<211> LENGTH: 438
<212> TYPE: PRT
<213> ORGANISM: Bacteroides thetaiotaomicron

<400> SEQUENCE: 15

Met Ala Thr Lys Glu Phe Phe Pro Gly Ile Glu Lys Ile Lys Phe Glu
1 5 10 15

Gly Lys Asp Ser Lys Asn Pro Met Ala Phe Arg Tyr Tyr Asp Ala Glu
20 25 30

Lys Val Ile Asn Gly Lys Lys Met Lys Asp Trp Leu Arg Phe Ala Met
35 40 45

Ala Trp Trp His Thr Leu Cys Ala Glu Gly Gly Asp Gln Phe Gly Gly
50 55 60

Gly Thr Lys Gln Phe Pro Trp Asn Gly Asn Ala Asp Ala Ile Gln Ala
65 70 75 80

Ala Lys Asp Lys Met Asp Ala Gly Phe Glu Phe Met Gln Lys Met Gly
85 90 95

Ile Glu Tyr Tyr Cys Phe His Asp Val Asp Leu Val Ser Glu Gly Ala
100 105 110

Ser Val Glu Glu Tyr Glu Ala Asn Leu Lys Glu Ile Val Ala Tyr Ala
115 120 125

Lys Gln Lys Gln Ala Glu Thr Gly Ile Lys Leu Leu Trp Gly Thr Ala
130 135 140

Asn Val Phe Gly His Ala Arg Tyr Met Asn Gly Ala Ala Thr Asn Pro
145 150 155 160

Asp Phe Asp Val Val Ala Arg Ala Ala Val Gln Ile Lys Asn Ala Ile
165 170 175

Asp Ala Thr Ile Glu Leu Gly Gly Glu Asn Tyr Val Phe Trp Gly Gly
180 185 190

Arg Glu Gly Tyr Met Ser Leu Leu Asn Thr Asp Gln Lys Arg Glu Lys
195 200 205

Glu His Leu Ala Gln Met Leu Thr Ile Ala Arg Asp Tyr Ala Arg Ala
210 215 220

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Arg Gly Phe Lys Gly Thr Phe Leu Ile Glu Pro Lys Pro Met Glu Pro
225                 230                 235                 240

Thr Lys His Gln Tyr Asp Val Asp Thr Glu Thr Val Ile Gly Phe Leu
                245                 250                 255

Lys Ala His Gly Leu Asp Lys Asp Phe Lys Val Asn Ile Glu Val Asn
            260                 265                 270

His Ala Thr Leu Ala Gly His Thr Phe Glu His Glu Leu Ala Val Ala
        275                 280                 285

Val Asp Asn Gly Met Leu Gly Ser Ile Asp Ala Asn Arg Gly Asp Tyr
    290                 295                 300

Gln Asn Gly Trp Asp Thr Asp Gln Phe Pro Ile Asp Asn Tyr Glu Leu
305                 310                 315                 320

Thr Gln Ala Met Met Gln Ile Ile Arg Asn Gly Gly Leu Gly Thr Gly
                325                 330                 335

Gly Thr Asn Phe Asp Ala Lys Thr Arg Arg Asn Ser Thr Asp Leu Glu
            340                 345                 350

Asp Ile Phe Ile Ala His Ile Ala Gly Met Asp Ala Met Ala Arg Ala
        355                 360                 365

Leu Glu Ser Ala Ala Ala Leu Leu Asp Glu Ser Pro Tyr Lys Lys Met
    370                 375                 380

Leu Ala Asp Arg Tyr Ala Ser Phe Asp Gly Gly Lys Gly Lys Glu Phe
385                 390                 395                 400

Glu Asp Gly Lys Leu Thr Leu Glu Asp Val Val Ala Tyr Ala Lys Thr
                405                 410                 415

Lys Gly Glu Pro Lys Gln Thr Ser Gly Lys Gln Glu Leu Tyr Glu Ala
            420                 425                 430

Ile Leu Asn Met Tyr Cys
        435
```

<210> SEQ ID NO 16
<211> LENGTH: 1317
<212> TYPE: DNA
<213> ORGANISM: Bacteroides thetaiotaomicron

<400> SEQUENCE: 16

```
atggcaacaa aagaattttt tccgggaatt gaaaagatta aatttgaagg taaagatagt     60

aagaacccga tggcattccg ttattacgat gcagagaagg tgattaatgg taaaaagatg    120

aaggattggc tgagattcgc tatggcatgg tggcacacat tgtgcgctga aggtggtgat    180

cagttcggtg gcggaacaaa gcaattccca tggaatggta atgcagatgc tatacaggca    240

gcaaaagata agatggatgc aggatttgaa ttcatgcaga agatgggtat cgaatactat    300

tgcttccatg acgtagactt ggtttcggaa ggtgccagtg tagaagaata cgaagctaac    360

ctgaaagaaa tcgtagctta tgcaaaacag aaacaggcag aaaccggtat caaactactg    420

tggggtactg ctaatgtatt cggtcacgcc cgctatatga acggtgcagc taccaatcct    480

gacttcgatg tagtagctcg tgctgctgtt cagatcaaaa atgcgattga tgcaacgatt    540

gaacttggcg gagagaatta tgtgttttgg ggtggtcgtg aaggctatat gtctcttctg    600

aacacagatc agaaacgtga aaaagaacac cttgcacaga tgttgacgat tgctcgtgac    660

tatgcccgtg cccgtggttt caaaggtact ttcctgatcg aaccgaaacc gatggaaccg    720

actaaacatc aatatgacgt agatacggaa actgtaatcg gcttcctgaa agctcatggt    780
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ctggataagg atttcaaagt aaatatcgag gtgaatcacg caactttggc aggtcacact 840

ttcgagcatg aattggctgt agctgtagac aatggtatgt tgggctcaat tgacgccaat 900

cgtggtgact atcagaatgg ctgggataca gaccaattcc cgatcgacaa ttatgaactg 960

actcaggcta tgatgcagat tatccgtaat ggtggtctcg gtaccggtgg tacgaacttt 1020

gatgctaaaa cccgtcgtaa ttctactgat ctggaagata tctttattgc tcacatcgca 1080

ggtatggacg ctatggcccg tgcactcgaa agtgcagcgg ctctgctcga cgaatctccc 1140

tataagaaga tgctggctga ccgttatgct tcatttgatg ggggcaaagg taaagaattt 1200

gaagacggca agctgactct ggaggatgtg gttgcttatg caaaaacaaa aggcgaaccg 1260

aaacagacta gcggcaagca agaactttat gaggcaattc tgaatatgta ttgctaa 1317

<210> SEQ ID NO 17
<211> LENGTH: 258
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 17

Met Ala Ala Gly Val Pro Lys Ile Asp Ala Leu Glu Ser Leu Gly Asn
1 5 10 15

Pro Leu Glu Asp Ala Lys Arg Ala Ala Ala Tyr Arg Ala Val Asp Glu
20 25 30

Asn Leu Lys Phe Asp Asp His Lys Ile Ile Gly Ile Gly Ser Gly Ser
35 40 45

Thr Val Val Tyr Val Ala Glu Arg Ile Gly Gln Tyr Leu His Asp Pro
50 55 60

Lys Phe Tyr Glu Val Ala Ser Lys Phe Ile Cys Ile Pro Thr Gly Phe
65 70 75 80

Gln Ser Arg Asn Leu Ile Leu Asp Asn Lys Leu Gln Leu Gly Ser Ile
85 90 95

Glu Gln Tyr Pro Arg Ile Asp Ile Ala Phe Asp Gly Ala Asp Glu Val
100 105 110

Asp Glu Asn Leu Gln Leu Ile Lys Gly Gly Gly Ala Cys Leu Phe Gln
115 120 125

Glu Lys Leu Val Ser Thr Ser Ala Lys Thr Phe Ile Val Val Ala Asp
130 135 140

Ser Arg Lys Lys Ser Pro Lys His Leu Gly Lys Asn Trp Arg Gln Gly
145 150 155 160

Val Pro Ile Glu Ile Val Pro Ser Ser Tyr Val Arg Val Lys Asn Asp
165 170 175

Leu Leu Glu Gln Leu His Ala Glu Lys Val Asp Ile Arg Gln Gly Gly
180 185 190

Ser Ala Lys Ala Gly Pro Val Val Thr Asp Asn Asn Asn Phe Ile Ile
195 200 205

Asp Ala Asp Phe Gly Glu Ile Ser Asp Pro Arg Lys Leu His Arg Glu
210 215 220

Ile Lys Leu Leu Val Gly Val Val Glu Thr Gly Leu Phe Ile Asp Asn
225 230 235 240

Ala Ser Lys Ala Tyr Phe Gly Asn Ser Asp Gly Ser Val Glu Val Thr
245 250 255

Glu Lys

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<210> SEQ ID NO 18
<211> LENGTH: 2467
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 18

ggatccaaga ccattattcc atcagaatgg aaaaaagttt aaaagatcac ggagattttg 60

ttcttctgag cttctgctgt ccttgaaaac aaattattcc gctggccgcc ccaaacaaaa 120

acaaccccga tttaataaca ttgtcacagt attagaaatt ttctttttac aaattaccat 180

ttccagctta ctacttccta taatcctcaa tcttcagcaa gcgacgcagg gaatagccgc 240

tgaggtgcat aactgtcact tttcaattcg gccaatgcaa tctcaggcgg acgaataagg 300

gggccctctc gagaaaaaca aaaggaggat gagattagta ctttaatgtt gtgttcagta 360

attcagagac agacaagaga ggtttccaac acaatgtctt tagactcata ctatcttggg 420

tttgatcttt cgacccaaca actgaaatgt ctcgccatta accaggacct aaaaattgtc 480

cattcagaaa cagtggaatt tgaaaaggat cttccgcatt atcacacaaa gaagggtgtc 540

tatatacacg gcgacactat cgaatgtccc gtagccatgt ggttaggggc tctagatctg 600

gttctctcga aatatcgcga ggctaaattt ccattgaaca aagttatggc cgtctcaggg 660

tcctgccagc agcacgggtc tgtctactgg tcctcccaag ccgaatctct gttagagcaa 720

ttgaataaga aaccggaaaa agatttattg cactacgtga gctctgtagc atttgcaagg 780

caaaccgccc ccaattggca agaccacagt actgcaaagc aatgtcaaga gtttgaagag 840

tgcataggtg ggcctgaaaa aatggctcaa ttaacagggt ccagagccca ttttagattt 900

actggtcctc aaattctgaa aattgcacaa ttagaaccag aagcttacga aaaaacaaag 960

accatttctt tagtgtctaa ttttttgact tctatcttag tgggccatct tgttgaatta 1020

gaggaggcag atgcctgtgg tatgaacctt tatgatatac gtgaaagaaa attcatgtat 1080

gagctactac atctaattga tagttcttct aaggataaaa ctatcagaca aaaattaatg 1140

agagcaccca tgaaaaattt gatagcgggt accatctgta aatattttat tgagaagtac 1200

ggtttcaata caaactgcaa ggtctctccc atgactgggg ataatttagc cactatatgt 1260

tctttacccc tgcggaagaa tgacgttctc gtttccctag gaacaagtac tacagttctt 1320

ctggtcaccg ataagtatca cccctctccg aactatcatc ttttcattca tccaactctg 1380

ccaaaccatt atatgggtat gatttgttat tgtaatggtt ctttggcaag ggagaggata 1440

agagacgagt taaacaaaga acgggaaaat aattatgaga agactaacga ttggactctt 1500

tttaatcaag ctgtgctaga tgactcagaa agtagtgaaa atgaattagg tgtatatttt 1560

cctctggggg agatcgttcc tagcgtaaaa gccataaaca aaagggttat cttcaatcca 1620

aaaacgggta tgattgaaag agaggtggcc aagttcaaag acaagaggca cgatgccaaa 1680

aatattgtag aatcacaggc tttaagttgc agggtaagaa tatctcccct gctttcggat 1740

tcaaacgcaa gctcacaaca gagactgaac gaagatacaa tcgtgaagtt tgattacgat 1800

gaatctccgc tgcgggacta cctaaataaa aggccagaaa ggacttttt tgtaggtggg 1860

gcttctaaaa acgatgctat tgtgaagaag tttgctcaag tcattggtgc tacaaagggt 1920

aattttaggc tagaaacacc aaactcatgt gcccttggtg gttgttataa ggccatgtgg 1980

tcattgttat atgactctaa taaaattgca gttccttttg ataaatttct gaatgacaat 2040

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tttccatggc atgtaatgga aagcatatcc gatgtggata atgaaaattg gatcgctata 2100

attccaagat tgtcccctta agcgaactgg aaaagactct catctaaaat atgtttgaat 2160

aatttatcat gccctgacaa gtacacacaa acacagacac ataatataca tacatatata 2220

tatatcaccg ttattatgcg tgcacatgac aatgcccttg tatgtttcgt atactgtagc 2280

aagtagtcat cattttgttc cccgttcgga aaatgacaaa aagtaaaatc aataaatgaa 2340

gagtaaaaaa caatttatga aagggtgagc gaccagcaac gagagagaca aatcaaatta 2400

gcgctttcca gtgagaatat aagagagcat tgaaagagct aggttattgt taaatcatct 2460

cgagctc 2467

<210> SEQ ID NO 19
<211> LENGTH: 238
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 19

Met Val Lys Pro Ile Ile Ala Pro Ser Ile Leu Ala Ser Asp Phe Ala
1 5 10 15

Asn Leu Gly Cys Glu Cys His Lys Val Ile Asn Ala Gly Ala Asp Trp
20 25 30

Leu His Ile Asp Val Met Asp Gly His Phe Val Pro Asn Ile Thr Leu
35 40 45

Gly Gln Pro Ile Val Thr Ser Leu Arg Arg Ser Val Pro Arg Pro Gly
50 55 60

Asp Ala Ser Asn Thr Glu Lys Lys Pro Thr Ala Phe Phe Asp Cys His
65 70 75 80

Met Met Val Glu Asn Pro Glu Lys Trp Val Asp Asp Phe Ala Lys Cys
85 90 95

Gly Ala Asp Gln Phe Thr Phe His Tyr Glu Ala Thr Gln Asp Pro Leu
100 105 110

His Leu Val Lys Leu Ile Lys Ser Lys Gly Ile Lys Ala Ala Cys Ala
115 120 125

Ile Lys Pro Gly Thr Ser Val Asp Val Leu Phe Glu Leu Ala Pro His
130 135 140

Leu Asp Met Ala Leu Val Met Thr Val Glu Pro Gly Phe Gly Gly Gln
145 150 155 160

Lys Phe Met Glu Asp Met Met Pro Lys Val Glu Thr Leu Arg Ala Lys
165 170 175

Phe Pro His Leu Asn Ile Gln Val Asp Gly Gly Leu Gly Lys Glu Thr
180 185 190

Ile Pro Lys Ala Ala Lys Ala Gly Ala Asn Val Ile Val Ala Gly Thr
195 200 205

Ser Val Phe Thr Ala Ala Asp Pro His Asp Val Ile Ser Phe Met Lys
210 215 220

Glu Glu Val Ser Lys Glu Leu Arg Ser Arg Asp Leu Leu Asp
225 230 235

<210> SEQ ID NO 20
<211> LENGTH: 1328
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 20

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gttaggcact tacgtatctt gtatagtagg aatggctcgg tttatgtata ttaggagatc 60

aaaacgagaa aaaaatacca tatcgtatag tatagagagt ataaatataa gaaatgccgc 120

atatgtacaa ctaatctagc aaatctctag aacgcaattc cttcgagact tcttctttca 180

tgaaggagat aacatcgtgc gggtcagctg cagtgaaaac actggtacca gcgacaataa 240

cgttggcacc ggctttggcg gctttcggga tggtctcctt gcccaaacca ccatcgactt 300

ggatattcaa atgggggaac ttggctctca aagtttccac ttttggcatc atgtcttcca 360

tgaatttttg gcctccaaac ccaggttcca cagtcataac aagagccata tccaaatgag 420

gagctagttc aaataaaacg tcaacagaag taccaggttt gatggcgcat gcagctttga 480

tgcccttaga cttaatcaac ttaactaaat gcaaagggtc ttgtgtggcc tcgtagtgga 540

acgtaaattg gtcagcacca catttagcaa aatcgtcgac ccatttttca ggattttcaa 600

ccatcatgtg acaatcgaag aacgcagtgg gcttcttttc tgtgttgcta gcatcgccag 660

ggcgtggcac agaacgacgt agggaggtaa caattggttg gcccagagta atgtttggaa 720

caaaatggcc gtccatgaca tcgatatgta accaatctgc gccggcgttg atgaccttat 780

gacattcgca acccaagttg gcgaagtcag aagcaaggat actgggagct ataattggtt 840

tgaccatttt ttcttgtgtg tttacctcgc tcttggaatt agcaaatggc cttcttgcat 900

gaaattgtat cgagtttgct ttatttttct ttttacgggc ggattctttc tattctggct 960

ttcctataac agagatcatg aaagaagttc cagcttacgg atcaagaaag tacctataca 1020

tatacaaaaa tctgattact ttcccagctc gacttggata gctgttcttg ttttctcttg 1080

gcgacacatt ttttgtttct gaagccacgt cctgctttat aagaggacat ttaaagttgc 1140

aggacttgaa tgcaattacc ggaagaagca accaaccggc atggttcagc atacaataca 1200

catttgatta gaaaagcaga gaataaatag acatgatacc tctcttttta tcctctgcag 1260

cgtattattg tttattccac gcaggcatcg gtcgttggct gttgttatgt ctcagataag 1320

cgcgtttg 1328

<210> SEQ ID NO 21
<211> LENGTH: 680
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 21

Met Thr Gln Phe Thr Asp Ile Asp Lys Leu Ala Val Ser Thr Ile Arg
1 5 10 15

Ile Leu Ala Val Asp Thr Val Ser Lys Ala Asn Ser Gly His Pro Gly
20 25 30

Ala Pro Leu Gly Met Ala Pro Ala Ala His Val Leu Trp Ser Gln Met
35 40 45

Arg Met Asn Pro Thr Asn Pro Asp Trp Ile Asn Arg Asp Arg Phe Val
50 55 60

Leu Ser Asn Gly His Ala Val Ala Leu Leu Tyr Ser Met Leu His Leu
65 70 75 80

Thr Gly Tyr Asp Leu Ser Ile Glu Asp Leu Lys Gln Phe Arg Gln Leu
85 90 95

Gly Ser Arg Thr Pro Gly His Pro Glu Phe Glu Leu Pro Gly Val Glu
100 105 110

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Val Thr Thr Gly Pro Leu Gly Gln Gly Ile Ser Asn Ala Val Gly Met
        115                 120                 125

Ala Met Ala Gln Ala Asn Leu Ala Ala Thr Tyr Asn Lys Pro Gly Phe
    130                 135                 140

Thr Leu Ser Asp Asn Tyr Thr Tyr Val Phe Leu Gly Asp Gly Cys Leu
145                 150                 155                 160

Gln Glu Gly Ile Ser Ser Glu Ala Ser Ser Leu Ala Gly His Leu Lys
                165                 170                 175

Leu Gly Asn Leu Ile Ala Ile Tyr Asp Asp Asn Lys Ile Thr Ile Asp
            180                 185                 190

Gly Ala Thr Ser Ile Ser Phe Asp Glu Asp Val Ala Lys Arg Tyr Glu
        195                 200                 205

Ala Tyr Gly Trp Glu Val Leu Tyr Val Glu Asn Gly Asn Glu Asp Leu
    210                 215                 220

Ala Gly Ile Ala Lys Ala Ile Ala Gln Ala Lys Leu Ser Lys Asp Lys
225                 230                 235                 240

Pro Thr Leu Ile Lys Met Thr Thr Thr Ile Gly Tyr Gly Ser Leu His
                245                 250                 255

Ala Gly Ser His Ser Val His Gly Ala Pro Leu Lys Ala Asp Asp Val
            260                 265                 270

Lys Gln Leu Lys Ser Lys Phe Gly Phe Asn Pro Asp Lys Ser Phe Val
        275                 280                 285

Val Pro Gln Glu Val Tyr Asp His Tyr Gln Lys Thr Ile Leu Lys Pro
    290                 295                 300

Gly Val Glu Ala Asn Asn Lys Trp Asn Lys Leu Phe Ser Glu Tyr Gln
305                 310                 315                 320

Lys Lys Phe Pro Glu Leu Gly Ala Glu Leu Ala Arg Arg Leu Ser Gly
                325                 330                 335

Gln Leu Pro Ala Asn Trp Glu Ser Lys Leu Pro Thr Tyr Thr Ala Lys
            340                 345                 350

Asp Ser Ala Val Ala Thr Arg Lys Leu Ser Glu Thr Val Leu Glu Asp
        355                 360                 365

Val Tyr Asn Gln Leu Pro Glu Leu Ile Gly Gly Ser Ala Asp Leu Thr
    370                 375                 380

Pro Ser Asn Leu Thr Arg Trp Lys Glu Ala Leu Asp Phe Gln Pro Pro
385                 390                 395                 400

Ser Ser Gly Ser Gly Asn Tyr Ser Gly Arg Tyr Ile Arg Tyr Gly Ile
                405                 410                 415

Arg Glu His Ala Met Gly Ala Ile Met Asn Gly Ile Ser Ala Phe Gly
            420                 425                 430

Ala Asn Tyr Lys Pro Tyr Gly Gly Thr Phe Leu Asn Phe Val Ser Tyr
        435                 440                 445

Ala Ala Gly Ala Val Arg Leu Ser Ala Leu Ser Gly His Pro Val Ile
    450                 455                 460

Trp Val Ala Thr His Asp Ser Ile Gly Val Gly Glu Asp Gly Pro Thr
465                 470                 475                 480

His Gln Pro Ile Glu Thr Leu Ala His Phe Arg Ser Leu Pro Asn Ile
                485                 490                 495

Gln Val Trp Arg Pro Ala Asp Gly Asn Glu Val Ser Ala Ala Tyr Lys
            500                 505                 510

Asn Ser Leu Glu Ser Lys His Thr Pro Ser Ile Ile Ala Leu Ser Arg
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        515                 520                 525

Gln Asn Leu Pro Gln Leu Glu Gly Ser Ser Ile Glu Ser Ala Ser Lys
    530                 535                 540

Gly Gly Tyr Val Leu Gln Asp Val Ala Asn Pro Asp Ile Ile Leu Val
545                 550                 555                 560

Ala Thr Gly Ser Glu Val Ser Leu Ser Val Glu Ala Ala Lys Thr Leu
                565                 570                 575

Ala Ala Lys Asn Ile Lys Ala Arg Val Val Ser Leu Pro Asp Phe Phe
            580                 585                 590

Thr Phe Asp Lys Gln Pro Leu Glu Tyr Arg Leu Ser Val Leu Pro Asp
        595                 600                 605

Asn Val Pro Ile Met Ser Val Glu Val Leu Ala Thr Thr Cys Trp Gly
    610                 615                 620

Lys Tyr Ala His Gln Ser Phe Gly Ile Asp Arg Phe Gly Ala Ser Gly
625                 630                 635                 640

Lys Ala Pro Glu Val Phe Lys Phe Phe Gly Phe Thr Pro Glu Gly Val
                645                 650                 655

Ala Glu Arg Ala Gln Lys Thr Ile Ala Phe Tyr Lys Gly Asp Lys Leu
            660                 665                 670

Ile Ser Pro Leu Lys Lys Ala Phe
        675                 680


<210> SEQ ID NO 22
<211> LENGTH: 2046
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 22

atggcacagt tctccgacat tgataaactt gcggtttcca ctttaagatt actttccgtt     60

gaccaggtgg aaagcgcaca atctggccac ccaggtgcac cactaggatt ggcaccagtt    120

gcccatgtaa ttttcaagca actgcgctgt aaccctaaca atgaacattg gatcaataga    180

gacaggtttg ttctgtcgaa cggtcactca tgcgctcttc tgtactcaat gctccatcta    240

ttaggatacg attactctat cgaggacttg agacaattta gacaagtaaa ctcaaggaca    300

ccgggtcatc cagaattcca ctcagcggga gtggaaatca cttccggtcc gctaggccag    360

ggtatctcaa atgctgttgg tatggcaata gcgcaggcca actttgccgc cacttataac    420

gaggatggct ttcccatttc cgactcatat acgtttgcta ttgtagggga tggttgctta    480

caagagggtg tttcttcgga gacctcttcc ttagcgggac atctgcaatt gggtaacttg    540

attacgtttt atgacagtaa tagcatttcc attgacggta aaacctcgta ctcgttcgac    600

gaagatgttt tgaagcgata cgaggcatat ggttgggaag tcatggaagt cgataaagga    660

gacgacgata tggaatccat ttctagcgct ttggaaaagg caaaactatc gaaggacaag    720

ccaaccataa tcaaggtaac tactacaatt ggatttgggt ccctacaaca gggtactgct    780

ggtgttcatg ggtccgcttt gaaggcagat gatgttaaac agttgaagaa gaggtggggg    840

tttgacccaa ataaatcatt tgtagtacct caagaggtgt acgattatta taagaagact    900

gttgtggaac ccggtcaaaa acttaatgag gaatgggata ggatgtttga agaatacaaa    960

accaaatttc ccgagaaggg taaagaattg caaagaagat tgaatggtga gttaccggaa   1020

ggttgggaaa agcatttacc gaagtttact ccggacgacg atgctctggc aacaagaaag   1080
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acatcccagc aggtgctgac gaacatggtc caagttttgc ctgaattgat cggtggttct 1140

gccgatttga caccttcgaa tctgacaagg tgggaaggcg cggtagattt ccaacctccc 1200

attacccaac taggtaacta tgcaggaagg tacattagat acggtgtgag ggaacacgga 1260

atgggtgcca ttatgaacgg tatctctgcc tttggtgcaa actacaagcc ttacggtggt 1320

acctttttga acttcgtctc ttatgctgca ggagccgtta ggttagccgc cttgtctggt 1380

aatccagtca tttgggttgc aacacatgac tctatcgggc ttggtgagga tggtccaacg 1440

caccaaccta ttgaaactct ggctcacttg agggctattc caaacatgca tgtatggaga 1500

cctgctgatg gtaacgaaac ttctgctgcg tattattctg ctatcaaatc tggtcgaaca 1560

ccatctgttg tggctttatc acgacagaat cttcctcaat tggagcattc ctcttttgaa 1620

aaagccttga agggtggcta tgtgatccat gacgtggaga atcctgatat tatcctggtg 1680

tcaacaggat cagaagtctc catttctata gatgcagcca aaaaattgta cgatactaaa 1740

aaaatcaaag caagagttgt ttccctgcca gacttttata cttttgacag gcaaagtgaa 1800

gaatacagat tctctgttct accagacggt gttccgatca tgtcctttga agtattggct 1860

acttcaagct ggggtaagta tgctcatcaa tcgttcggac tcgacgaatt tggtcgttca 1920

ggcaaggggc ctgaaattta caaattgttc gatttcacag cggacggtgt tgcgtcaagg 1980

gctgaaaaga caatcaatta ctacaaagga aagcagttgc tttctcctat gggaagagct 2040

ttctaa 2046

<210> SEQ ID NO 23
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 23

Met Ser Glu Pro Ala Gln Lys Lys Gln Lys Val Ala Asn Asn Ser Leu
1 5 10 15

Glu Gln Leu Lys Ala Ser Gly Thr Val Val Val Ala Asp Thr Gly Asp
20 25 30

Phe Gly Ser Ile Ala Lys Phe Gln Pro Gln Asp Ser Thr Thr Asn Pro
35 40 45

Ser Leu Ile Leu Ala Ala Ala Lys Gln Pro Thr Tyr Ala Lys Leu Ile
50 55 60

Asp Val Ala Val Glu Tyr Gly Lys Lys His Gly Lys Thr Thr Glu Glu
65 70 75 80

Gln Val Glu Asn Ala Val Asp Arg Leu Leu Val Glu Phe Gly Lys Glu
85 90 95

Ile Leu Lys Ile Val Pro Gly Arg Val Ser Thr Glu Val Asp Ala Arg
100 105 110

Leu Ser Phe Asp Thr Gln Ala Thr Ile Glu Lys Ala Arg His Ile Ile
115 120 125

Lys Leu Phe Glu Gln Glu Gly Val Ser Lys Glu Arg Val Leu Ile Lys
130 135 140

Ile Ala Ser Thr Trp Glu Gly Ile Gln Ala Ala Lys Glu Leu Glu Glu
145 150 155 160

Lys Asp Gly Ile His Cys Asn Leu Thr Leu Leu Phe Ser Phe Val Gln
165 170 175

Ala Val Ala Cys Ala Glu Ala Gln Val Thr Leu Ile Ser Pro Phe Val

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            180                 185                 190

Gly Arg Ile Leu Asp Trp Tyr Lys Ser Ser Thr Gly Lys Asp Tyr Lys
        195                 200                 205

Gly Glu Ala Asp Pro Gly Val Ile Ser Val Lys Lys Ile Tyr Asn Tyr
    210                 215                 220

Tyr Lys Lys Tyr Gly Tyr Lys Thr Ile Val Met Gly Ala Ser Phe Arg
225                 230                 235                 240

Ser Thr Asp Glu Ile Lys Asn Leu Ala Gly Val Asp Tyr Leu Thr Ile
                245                 250                 255

Ser Pro Ala Leu Leu Asp Lys Leu Met Asn Ser Thr Glu Pro Phe Pro
            260                 265                 270

Arg Val Leu Asp Pro Val Ser Ala Lys Lys Glu Ala Gly Asp Lys Ile
        275                 280                 285

Ser Tyr Ile Ser Asp Glu Ser Lys Phe Arg Phe Asp Leu Asn Glu Asp
    290                 295                 300

Ala Met Ala Thr Glu Lys Leu Ser Glu Gly Ile Arg Lys Phe Ser Ala
305                 310                 315                 320

Asp Ile Val Thr Leu Phe Asp Leu Ile Glu Lys Lys Val Thr Ala
                325                 330                 335


<210> SEQ ID NO 24
<211> LENGTH: 2046
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 24

atggcacagt tctccgacat tgataaactt gcggtttcca ctttaagatt actttccgtt     60

gaccaggtgg aaagcgcaca atctggccac ccaggtgcac cactaggatt ggcaccagtt    120

gcccatgtaa ttttcaagca actgcgctgt aaccctaaca atgaacattg gatcaataga    180

gacaggtttg ttctgtcgaa cggtcactca tgcgctcttc tgtactcaat gctccatcta    240

ttaggatacg attactctat cgaggacttg agacaattta gacaagtaaa ctcaaggaca    300

ccgggtcatc cagaattcca ctcagcggga gtggaaatca cttccggtcc gctaggccag    360

ggtatctcaa atgctgttgg tatggcaata gcgcaggcca actttgccgc cacttataac    420

gaggatggct ttcccatttc cgactcatat acgtttgcta ttgtagggga tggttgctta    480

caagagggtg tttcttcgga gacctcttcc ttagcgggac atctgcaatt gggtaacttg    540

attacgtttt atgacagtaa tagcatttcc attgacggta aaacctcgta ctcgttcgac    600

gaagatgttt tgaagcgata cgaggcatat ggttgggaag tcatggaagt cgataaagga    660

gacgacgata tggaatccat ttctagcgct ttggaaaagg caaaactatc gaaggacaag    720

ccaaccataa tcaaggtaac tactacaatt ggatttgggt ccctacaaca gggtactgct    780

ggtgttcatg ggtccgcttt gaaggcagat gatgttaaac agttgaagaa gaggtggggg    840

tttgacccaa ataaatcatt tgtagtacct caagaggtgt acgattatta taagaagact    900

gttgtggaac ccggtcaaaa acttaatgag gaatgggata ggatgtttga agaatacaaa    960

accaaatttc ccgagaaggg taaagaattg caaagaagat tgaatggtga gttaccggaa   1020

ggttgggaaa agcatttacc gaagtttact ccggacgacg atgctctggc aacaagaaag   1080

acatcccagc aggtgctgac gaacatggtc caagttttgc ctgaattgat cggtggttct   1140

gccgatttga caccttcgaa tctgacaagg tgggaaggcg cggtagattt ccaacctccc   1200
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attacccaac taggtaacta tgcaggaagg tacattagat acggtgtgag ggaacacgga 1260

atgggtgcca ttatgaacgg tatctctgcc tttggtgcaa actacaagcc ttacggtggt 1320

acctttttga acttcgtctc ttatgctgca ggagccgtta ggttagccgc cttgtctggt 1380

aatccagtca tttgggttgc aacacatgac tctatcgggc ttggtgagga tggtccaacg 1440

caccaaccta ttgaaactct ggctcacttg agggctattc caaacatgca tgtatggaga 1500

cctgctgatg gtaacgaaac ttctgctgcg tattattctg ctatcaaatc tggtcgaaca 1560

ccatctgttg tggctttatc acgacagaat cttcctcaat tggagcattc ctcttttgaa 1620

aaagccttga agggtggcta tgtgatccat gacgtggaga atcctgatat tatcctggtg 1680

tcaacaggat cagaagtctc catttctata gatgcagcca aaaaattgta cgatactaaa 1740

aaaatcaaag caagagttgt ttccctgcca gacttttata cttttgacag gcaaagtgaa 1800

gaatacagat tctctgttct accagacggt gttccgatca tgtcctttga agtattggct 1860

acttcaagct ggggtaagta tgctcatcaa tcgttcggac tcgacgaatt tggtcgttca 1920

ggcaaggggc ctgaaattta caaattgttc gatttcacag cggacggtgt tgcgtcaagg 1980

gctgaaaaga caatcaatta ctacaaagga aagcagttgc tttctcctat gggaagagct 2040

ttctaa 2046

<210> SEQ ID NO 25
<211> LENGTH: 600
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 25

Met Leu Cys Ser Val Ile Gln Arg Gln Thr Arg Glu Val Ser Asn Thr
1 5 10 15

Met Ser Leu Asp Ser Tyr Tyr Leu Gly Phe Asp Leu Ser Thr Gln Gln
20 25 30

Leu Lys Cys Leu Ala Ile Asn Gln Asp Leu Lys Ile Val His Ser Glu
35 40 45

Thr Val Glu Phe Glu Lys Asp Leu Pro His Tyr His Thr Lys Lys Gly
50 55 60

Val Tyr Ile His Gly Asp Thr Ile Glu Cys Pro Val Ala Met Trp Leu
65 70 75 80

Glu Ala Leu Asp Leu Val Leu Ser Lys Tyr Arg Glu Ala Lys Phe Pro
85 90 95

Leu Asn Lys Val Met Ala Val Ser Gly Ser Cys Gln Gln His Gly Ser
100 105 110

Val Tyr Trp Ser Ser Gln Ala Glu Ser Leu Leu Glu Gln Leu Asn Lys
115 120 125

Lys Pro Glu Lys Asp Leu Leu His Tyr Val Ser Ser Val Ala Phe Ala
130 135 140

Arg Gln Thr Ala Pro Asn Trp Gln Asp His Ser Thr Ala Lys Gln Cys
145 150 155 160

Gln Glu Phe Glu Glu Cys Ile Gly Gly Pro Glu Lys Met Ala Gln Leu
165 170 175

Thr Gly Ser Arg Ala His Phe Arg Phe Thr Gly Pro Gln Ile Leu Lys
180 185 190

Ile Ala Gln Leu Glu Pro Glu Ala Tyr Glu Lys Thr Lys Thr Ile Ser

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        195                 200                 205

Leu Val Ser Asn Phe Leu Thr Ser Ile Leu Val Gly His Leu Val Glu
    210                 215                 220

Leu Glu Glu Ala Asp Ala Cys Gly Met Asn Leu Tyr Asp Ile Arg Glu
225                 230                 235                 240

Arg Lys Phe Ser Asp Glu Leu Leu His Leu Ile Asp Ser Ser Ser Lys
                245                 250                 255

Asp Lys Thr Ile Arg Gln Lys Leu Met Arg Ala Pro Met Lys Asn Leu
            260                 265                 270

Ile Ala Gly Thr Ile Cys Lys Tyr Phe Ile Glu Lys Tyr Gly Phe Asn
        275                 280                 285

Thr Asn Cys Lys Val Ser Pro Met Thr Gly Asp Asn Leu Ala Thr Ile
    290                 295                 300

Cys Ser Leu Pro Leu Arg Lys Asn Asp Val Leu Val Ser Leu Gly Thr
305                 310                 315                 320

Ser Thr Thr Val Leu Leu Val Thr Asp Lys Tyr His Pro Ser Pro Asn
                325                 330                 335

Tyr His Leu Phe Ile His Pro Thr Leu Pro Asn His Tyr Met Gly Met
            340                 345                 350

Ile Cys Tyr Cys Asn Gly Ser Leu Ala Arg Glu Arg Ile Arg Asp Glu
        355                 360                 365

Leu Asn Lys Glu Arg Glu Asn Asn Tyr Glu Lys Thr Asn Asp Trp Thr
    370                 375                 380

Leu Phe Asn Gln Ala Val Leu Asp Asp Ser Glu Ser Ser Glu Asn Glu
385                 390                 395                 400

Leu Gly Val Tyr Phe Pro Leu Gly Glu Ile Val Pro Ser Val Lys Ala
                405                 410                 415

Ile Asn Lys Arg Val Ile Phe Asn Pro Lys Thr Gly Met Ile Glu Arg
            420                 425                 430

Glu Val Ala Lys Phe Lys Asp Lys Arg His Asp Ala Lys Asn Ile Val
        435                 440                 445

Glu Ser Gln Ala Leu Ser Cys Arg Val Arg Ile Ser Pro Leu Leu Ser
    450                 455                 460

Asp Ser Asn Ala Ser Ser Gln Gln Arg Leu Asn Glu Asp Thr Ile Val
465                 470                 475                 480

Lys Phe Asp Tyr Asp Glu Ser Pro Leu Arg Asp Tyr Leu Asn Lys Arg
                485                 490                 495

Pro Glu Arg Thr Phe Phe Val Gly Gly Ala Ser Lys Asn Asp Ala Ile
            500                 505                 510

Val Lys Lys Phe Ala Gln Val Ile Gly Ala Thr Lys Gly Asn Phe Arg
        515                 520                 525

Leu Glu Thr Pro Asn Ser Cys Ala Leu Gly Gly Cys Tyr Lys Ala Met
    530                 535                 540

Trp Ser Leu Leu Tyr Asp Ser Asn Lys Ile Ala Val Pro Phe Asp Lys
545                 550                 555                 560

Phe Leu Asn Asp Asn Phe Pro Trp His Val Met Glu Ser Ile Ser Asp
                565                 570                 575

Val Asp Asn Glu Asn Trp Asp Arg Tyr Asn Ser Lys Ile Val Pro Leu
            580                 585                 590

Ser Glu Leu Glu Lys Thr Leu Ile
        595                 600
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<210> SEQ ID NO 26
<211> LENGTH: 2467
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 26

ggatccaaga ccattattcc atcagaatgg aaaaaagttt aaaagatcac ggagattttg 60

ttcttctgag cttctgctgt ccttgaaaac aaattattcc gctggccgcc ccaaacaaaa 120

acaaccccga tttaataaca ttgtcacagt attagaaatt ttctttttac aaattaccat 180

ttccagctta ctacttccta taatcctcaa tcttcagcaa gcgacgcagg gaatagccgc 240

tgaggtgcat aactgtcact tttcaattcg gccaatgcaa tctcaggcgg acgaataagg 300

gggccctctc gagaaaaaca aaaggaggat gagattagta ctttaatgtt gtgttcagta 360

attcagagac agacaagaga ggtttccaac acaatgtctt tagactcata ctatcttggg 420

tttgatcttt cgacccaaca actgaaatgt ctcgccatta accaggacct aaaaattgtc 480

cattcagaaa cagtggaatt tgaaaaggat cttccgcatt atcacacaaa gaagggtgtc 540

tatatacacg gcgacactat cgaatgtccc gtagccatgt ggttaggggc tctagatctg 600

gttctctcga aatatcgcga ggctaaattt ccattgaaca aagttatggc cgtctcaggg 660

tcctgccagc agcacgggtc tgtctactgg tcctcccaag ccgaatctct gttagagcaa 720

ttgaataaga aaccggaaaa agatttattg cactacgtga gctctgtagc atttgcaagg 780

caaaccgccc ccaattggca agaccacagt actgcaaagc aatgtcaaga gtttgaagag 840

tgcataggtg ggcctgaaaa aatggctcaa ttaacagggt ccagagccca ttttagattt 900

actggtcctc aaattctgaa aattgcacaa ttagaaccag aagcttacga aaaaacaaag 960

accatttctt tagtgtctaa ttttttgact tctatcttag tgggccatct tgttgaatta 1020

gaggaggcag atgcctgtgg tatgaacctt tatgatatac gtgaaagaaa attcatgtat 1080

gagctactac atctaattga tagttcttct aaggataaaa ctatcagaca aaaattaatg 1140

agagcaccca tgaaaaattt gatagcgggt accatctgta aatattttat tgagaagtac 1200

ggtttcaata caaactgcaa ggtctctccc atgactgggg ataatttagc cactatatgt 1260

tctttacccc tgcggaagaa tgacgttctc gtttccctag gaacaagtac tacagttctt 1320

ctggtcaccg ataagtatca cccctctccg aactatcatc ttttcattca tccaactctg 1380

ccaaaccatt atatgggtat gatttgttat tgtaatggtt ctttggcaag ggagaggata 1440

agagacgagt taaacaaaga acgggaaaat aattatgaga agactaacga ttggactctt 1500

tttaatcaag ctgtgctaga tgactcagaa agtagtgaaa atgaattagg tgtatatttt 1560

cctctggggg agatcgttcc tagcgtaaaa gccataaaca aaagggttat cttcaatcca 1620

aaaacgggta tgattgaaag agaggtggcc aagttcaaag acaagaggca cgatgccaaa 1680

aatattgtag aatcacaggc tttaagttgc agggtaagaa tatctcccct gctttcggat 1740

tcaaacgcaa gctcacaaca gagactgaac gaagatacaa tcgtgaagtt tgattacgat 1800

gaatctccgc tgcgggacta cctaaataaa aggccagaaa ggacttttt tgtaggtggg 1860

gcttctaaaa acgatgctat tgtgaagaag tttgctcaag tcattggtgc tacaaagggt 1920

aattttaggc tagaaacacc aaactcatgt gcccttggtg gttgttataa ggccatgtgg 1980

tcattgttat atgactctaa taaaattgca gttccttttg ataaatttct gaatgacaat 2040

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tttccatggc atgtaatgga aagcatatcc gatgtggata atgaaaattg gatcgctata 2100

attccaagat tgtcccctta agcgaactgg aaaagactct catctaaaat atgtttgaat 2160

aatttatcat gccctgacaa gtacacacaa acacagacac ataatataca tacatatata 2220

tatatcaccg ttattatgcg tgcacatgac aatgcccttg tatgtttcgt atactgtagc 2280

aagtagtcat cattttgttc cccgttcgga aaatgacaaa aagtaaaatc aataaatgaa 2340

gagtaaaaaa caatttatga aagggtgagc gaccagcaac gagagagaca aatcaaatta 2400

gcgctttcca gtgagaatat aagagagcat tgaaagagct aggttattgt taaatcatct 2460

cgagctc 2467

<210> SEQ ID NO 27
<211> LENGTH: 494
<212> TYPE: PRT
<213> ORGANISM: Piromyces species

<400> SEQUENCE: 27

Met Lys Thr Val Ala Gly Ile Asp Leu Gly Thr Gln Ser Met Lys Val
1 5 10 15

Val Ile Tyr Asp Tyr Glu Lys Lys Glu Ile Ile Glu Ser Ala Ser Cys
20 25 30

Pro Met Glu Leu Ile Ser Glu Ser Asp Gly Thr Arg Glu Gln Thr Thr
35 40 45

Glu Trp Phe Asp Lys Gly Leu Glu Val Cys Phe Gly Lys Leu Ser Ala
50 55 60

Asp Asn Lys Lys Thr Ile Glu Ala Ile Gly Ile Ser Gly Gln Leu His
65 70 75 80

Gly Phe Val Pro Leu Asp Ala Asn Gly Lys Ala Leu Tyr Asn Ile Lys
85 90 95

Leu Trp Cys Asp Thr Ala Thr Val Glu Glu Cys Lys Ile Ile Thr Asp
100 105 110

Ala Ala Gly Gly Asp Lys Ala Val Ile Asp Ala Leu Gly Asn Leu Met
115 120 125

Leu Thr Gly Phe Thr Ala Pro Lys Ile Leu Trp Leu Lys Arg Asn Lys
130 135 140

Pro Glu Ala Phe Ala Asn Leu Lys Tyr Ile Met Leu Pro His Asp Tyr
145 150 155 160

Leu Asn Trp Lys Leu Thr Gly Asp Tyr Val Met Glu Tyr Gly Asp Ala
165 170 175

Ser Gly Thr Ala Leu Phe Asp Ser Lys Asn Arg Cys Trp Ser Lys Lys
180 185 190

Ile Cys Asp Ile Ile Asp Pro Lys Leu Leu Asp Leu Leu Pro Lys Leu
195 200 205

Ile Glu Pro Ser Ala Pro Ala Gly Lys Val Asn Asp Glu Ala Ala Lys
210 215 220

Ala Tyr Gly Ile Pro Ala Gly Ile Pro Val Ser Ala Gly Gly Gly Asp
225 230 235 240

Asn Met Met Gly Ala Val Gly Thr Gly Thr Val Ala Asp Gly Phe Leu
245 250 255

Thr Met Ser Met Gly Thr Ser Gly Thr Leu Tyr Gly Tyr Ser Asp Lys
260 265 270

-continued

```
Pro Ile Ser Asp Pro Ala Asn Gly Leu Ser Gly Phe Cys Ser Ser Thr
        275                 280                 285

Gly Gly Trp Leu Pro Leu Leu Cys Thr Met Asn Cys Thr Val Ala Thr
    290                 295                 300

Glu Phe Val Arg Asn Leu Phe Gln Met Asp Ile Lys Glu Leu Asn Val
305                 310                 315                 320

Glu Ala Ala Lys Ser Pro Cys Gly Ser Glu Gly Val Leu Val Ile Pro
                325                 330                 335

Phe Phe Asn Gly Glu Arg Thr Pro Asn Leu Pro Asn Gly Arg Ala Ser
            340                 345                 350

Ile Thr Gly Leu Thr Ser Ala Asn Thr Ser Arg Ala Asn Ile Ala Arg
        355                 360                 365

Ala Ser Phe Glu Ser Ala Val Phe Ala Met Arg Gly Gly Leu Asp Ala
    370                 375                 380

Phe Arg Lys Leu Gly Phe Gln Pro Lys Glu Ile Arg Leu Ile Gly Gly
385                 390                 395                 400

Gly Ser Lys Ser Asp Leu Trp Arg Gln Ile Ala Ala Asp Ile Met Asn
                405                 410                 415

Leu Pro Ile Arg Val Pro Leu Leu Glu Glu Ala Ala Ala Leu Gly Gly
            420                 425                 430

Ala Val Gln Ala Leu Trp Cys Leu Lys Asn Gln Ser Gly Lys Cys Asp
        435                 440                 445

Ile Val Glu Leu Cys Lys Glu His Ile Lys Ile Asp Glu Ser Lys Asn
    450                 455                 460

Ala Asn Pro Ile Ala Glu Asn Val Ala Val Tyr Asp Lys Ala Tyr Asp
465                 470                 475                 480

Glu Tyr Cys Lys Val Val Asn Thr Leu Ser Pro Leu Tyr Ala
                485                 490
```

<210> SEQ ID NO 28
<211> LENGTH: 2041
<212> TYPE: DNA
<213> ORGANISM: Piromyces sp.

<400> SEQUENCE: 28

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attatataaa ataactttaa ataaaacaat ttttatttgt ttatttaatt attcaaaaaa     60

aattaaagta aaagaaaaat aatacagtag aacaatagta ataatatcaa aatgaagact    120

gttgctggta ttgatcttgg aactcaaagt atgaaagtcg ttatttacga ctatgaaaag    180

aaagaaatta ttgaaagtgc tagctgtcca atggaattga tttccgaaag tgacggtacc    240

cgtgaacaaa ccactgaatg gtttgacaag ggtcttgaag tttgttttgg taagcttagt    300

gctgataaca aaaagactat tgaagctatt ggtatttctg gtcaattaca cggttttgtt    360

cctcttgatg ctaacggtaa ggctttatac aacatcaaac tttggtgtga tactgctacc    420

gttgaagaat gtaagattat cactgatgct gccggtggtg acaaggctgt tattgatgcc    480

cttggtaacc ttatgctcac cggtttcacc gctccaaaga tcctctggct caagcgcaac    540

aagccagaag ctttcgctaa cttaaagtac attatgcttc cacacgatta cttaaactgg    600

aagcttactg gtgattacgt tatggaatac ggtgatgcct ctggtaccgc tctcttcgat    660

tctaagaacc gttgctggtc taagaagatt tgcgatatca ttgacccaaa acttttagat    720

ttacttccaa agttaattga accaagcgct ccagctggta aggttaatga tgaagccgct    780
```

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aaggcttacg gtattccagc cggtattcca gtttccgctg gtggtggtga taacatgatg 840

ggtgctgttg gtactggtac tgttgctgat ggtttcctta ccatgtctat gggtacttct 900

ggtactcttt acggttacag tgacaagcca attagtgacc cagctaatgg tttaagtggt 960

ttctgttctt ctactggtgg atggcttcca ttactttgta ctatgaactg tactgttgcc 1020

actgaattcg ttcgtaacct cttccaaatg gatattaagg aacttaatgt tgaagctgcc 1080

aagtctccat gtggtagtga aggtgtttta gttattccat tcttcaatgg tgaaagaact 1140

ccaaacttac caaacggtcg tgctagtatt actggtctta cttctgctaa caccagccgt 1200

gctaacattg ctcgtgctag tttcgaatcc gccgttttcg ctatgcgtgg tggtttagat 1260

gctttccgta agttaggttt ccaaccaaag gaaattcgtc ttattggtgg tggttctaag 1320

tctgatctct ggagacaaat tgccgctgat atcatgaacc ttccaatcag agttccactt 1380

ttagaagaag ctgctgctct tggtggtgct gttcaagctt tatggtgtct taagaaccaa 1440

tctggtaagt gtgatattgt tgaactttgc aaagaacaca ttaagattga tgaatctaag 1500

aatgctaacc caattgccga aaatgttgct gtttacgaca aggcttacga tgaatactgc 1560

aaggttgtaa atactctttc tccattatat gcttaaattg ccaatgtaaa aaaaaatata 1620

atgccatata attgccttgt caatacactg ttcatgttca tataatcata ggacattgaa 1680

tttacaaggt ttatacaatt aatatctatt atcatattat tatacagcat ttcattttct 1740

aagattagac gaaacaattc ttggttcctt gcaatataca aaatttacat gaatttttag 1800

aatagtctcg tatttatgcc caataatcag gaaaattacc taatgctgga ttcttgttaa 1860

taaaaacaaa ataaataaat taaataaaca aataaaaatt ataagtaaat ataaatatat 1920

aagtaatata aaaaaaaagt aaataaataa ataaataaat aaaaattttt tgcaaatata 1980

taaataaata aataaaatat aaaaataatt tagcaaataa attaaaaaaa aaaaaaaaaa 2040

a 2041

<210> SEQ ID NO 29
<211> LENGTH: 327
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 29

Met Ser Ser Leu Val Thr Leu Asn Asn Gly Leu Lys Met Pro Leu Val
1 5 10 15

Gly Leu Gly Cys Trp Lys Ile Asp Lys Lys Val Cys Ala Asn Gln Ile
20 25 30

Tyr Glu Ala Ile Lys Leu Gly Tyr Arg Leu Phe Asp Gly Ala Cys Asp
35 40 45

Tyr Gly Asn Glu Lys Glu Val Gly Glu Gly Ile Arg Lys Ala Ile Ser
50 55 60

Glu Gly Leu Val Ser Arg Lys Asp Ile Phe Val Val Ser Lys Leu Trp
65 70 75 80

Asn Asn Phe His His Pro Asp His Val Lys Leu Ala Leu Lys Lys Thr
85 90 95

Leu Ser Asp Met Gly Leu Asp Tyr Leu Asp Leu Tyr Tyr Ile His Phe
100 105 110

Pro Ile Ala Phe Lys Tyr Val Pro Phe Glu Glu Lys Tyr Pro Pro Gly
115 120 125

-continued

```
Phe Tyr Thr Gly Ala Asp Asp Glu Lys Lys Gly His Ile Thr Glu Ala
    130                 135                 140

His Val Pro Ile Ile Asp Thr Tyr Arg Ala Leu Glu Glu Cys Val Asp
145                 150                 155                 160

Glu Gly Leu Ile Lys Ser Ile Gly Val Ser Asn Phe Gln Gly Ser Leu
                165                 170                 175

Ile Gln Asp Leu Leu Arg Gly Cys Arg Ile Lys Pro Val Ala Leu Gln
            180                 185                 190

Ile Glu His His Pro Tyr Leu Thr Gln Glu His Leu Val Glu Phe Cys
        195                 200                 205

Lys Leu His Asp Ile Gln Val Val Ala Tyr Ser Ser Phe Gly Pro Gln
    210                 215                 220

Ser Phe Ile Glu Met Asp Leu Gln Leu Ala Lys Thr Thr Pro Thr Leu
225                 230                 235                 240

Phe Glu Asn Asp Val Ile Lys Lys Val Ser Gln Asn His Pro Gly Ser
                245                 250                 255

Thr Thr Ser Gln Val Leu Leu Arg Trp Ala Thr Gln Arg Gly Ile Ala
            260                 265                 270

Val Ile Pro Lys Ser Ser Lys Lys Glu Arg Leu Leu Gly Asn Leu Glu
        275                 280                 285

Ile Glu Lys Lys Phe Thr Leu Thr Glu Gln Glu Leu Lys Asp Ile Ser
    290                 295                 300

Ala Leu Asn Ala Asn Ile Arg Phe Asn Asp Pro Trp Thr Trp Leu Asp
305                 310                 315                 320

Gly Lys Phe Pro Thr Phe Ala
                325
```

<210> SEQ ID NO 30
<211> LENGTH: 984
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 30

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atgtcttcac tggttactct taataacggt ctgaaaatgc ccctagtcgg cttagggtgc     60

tggaaaattg acaaaaaagt ctgtgcgaat caaatttatg aagctatcaa attaggctac    120

cgtttattcg atggtgcttg cgactacggc aacgaaaagg aagttggtga aggtatcagg    180

aaagccatct ccgaaggtct tgtttctaga aaggatatat ttgttgtttc aaagttatgg    240

aacaattttc accatcctga tcatgtaaaa ttagctttaa agaagacctt aagcgatatg    300

ggacttgatt atttagacct gtattatatt cacttcccaa tcgccttcaa atatgttcca    360

tttgaagaga aataccctcc aggattctat acgggcgcag atgacgagaa gaaaggtcac    420

atcaccgaag cacatgtacc aatcatagat acgtaccggg ctctggaaga atgtgttgat    480

gaaggcttga ttaagtctat tggtgtttcc aactttcagg gaagcttgat tcaagattta    540

ttacgtggtt gtagaatcaa gcccgtggct ttgcaaattg aacaccatcc ttatttgact    600

caagaacacc tagttgagtt ttgtaaatta cacgatatcc aagtagttgc ttactcctcc    660

ttcggtcctc aatcattcat tgagatggac ttacagttgg caaaaaccac gccaactctg    720

ttcgagaatg atgtaatcaa gaaggtctca caaaaccatc caggcagtac cacttcccaa    780

gtattgctta gatgggcaac tcagagaggc attgccgtca ttccaaaatc ttccaagaag    840

gaaaggttac ttggcaacct agaaatcgaa aaaaagttca ctttaacgga gcaagaattg    900
```

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aaggatattt ctgcactaaa tgccaacatc agatttaatg atccatggac ctggttggat 960

ggtaaattcc ccacttttgc ctga 984

<210> SEQ ID NO 31
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 31

gactagtcga gtttatcatt atcaatactg c 31

<210> SEQ ID NO 32
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 32

ctcataatca ggtactgata acattttgtt tgtttatgtg tgtttattc 49

<210> SEQ ID NO 33
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 33

gaataaacac acataaacaa acaaaatgtt atcagtacct gattatgag 49

<210> SEQ ID NO 34
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 34

aatcataaat cataagaaat tcgcttactt taagaatgcc ttagtcat 48

<210> SEQ ID NO 35
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 35

atgactaagg cattcttaaa gtaagcgaat ttcttatgat ttatgatt 48

<210> SEQ ID NO 36
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 36

cactagtctc gagtgtggaa gaacgattac aacagg 36

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<210> SEQ ID NO 37
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 37

cgagctcgtg ggtgtattgg attataggaa g 31

<210> SEQ ID NO 38
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 38

ttgggctgtt tcaactaaat tcatttttag gctggtatct tgattcta 48

<210> SEQ ID NO 39
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 39

tagaatcaag ataccagcct aaaaatgaat ttagttgaaa cagcccaa 48

<210> SEQ ID NO 40
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 40

aatcataaat cataagaaat tcgctctaat atttgattgc ttgcccag 48

<210> SEQ ID NO 41
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 41

ctgggcaagc aatcaaatat tagagcgaat ttcttatgat ttatgatt 48

<210> SEQ ID NO 42
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 42

tgagctcgtg tggaagaacg attacaacag g 31

<210> SEQ ID NO 43
<211> LENGTH: 28

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 43

acgcgtcgac tcgtaggaac aatttcgg 28

<210> SEQ ID NO 44
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 44

cttcttgttt taatgcttct agcatttttt gattaaaatt aaaaaaactt 50

<210> SEQ ID NO 45
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 45

aagttttttt aattttaatc aaaaaatgct agaagcatta aaacaagaag 50

<210> SEQ ID NO 46
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 46

ggtatatatt taagagcgat ttgtttactt gcgaactgca tgatcc 46

<210> SEQ ID NO 47
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 47

ggatcatgca gttcgcaagt aaacaaatcg ctcttaaata tatacc 46

<210> SEQ ID NO 48
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 48

cgcagtcgac cttttaaaca gttgatgaga acc 33

<210> SEQ ID NO 49
<211> LENGTH: 676
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: promoter

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<400> SEQUENCE: 49

tcgagtttat cattatcaat actgccattt caaagaatac gtaaataatt aatagtagtg 60

attttcctaa ctttatttag tcaaaaaatt agccttttaa ttctgctgta acccgtacat 120

gcccaaaata gggggcgggt tacacagaat atataacatc gtaggtgtct gggtgaacag 180

tttattcctg gcatccacta aatataatgg agcccgcttt ttaagctggc atccagaaaa 240

aaaaagaatc ccagcaccaa aatattgttt tcttcaccaa ccatcagttc ataggtccat 300

tctcttagcg caactacaga gaacaggggc acaaacaggc aaaaaacggg cacaacctca 360

atggagtgat gcaacctgcc tggagtaaat gatgacacaa ggcaattgac ccacgcatgt 420

atctatctca ttttcttaca ccttctatta ccttctgctc tctctgattt ggaaaaagct 480

gaaaaaaaag gttgaaacca gttccctgaa attattcccc tacttgacta ataagtatat 540

aaagacggta ggtattgatt gtaattctgt aaatctattt cttaaacttc ttaaattcta 600

cttttatagt tagtcttttt tttagtttta aaacaccaag aacttagttt cgaataaaca 660

cacataaaca aacaaa 676

<210> SEQ ID NO 50
<211> LENGTH: 326
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: terminator

<400> SEQUENCE: 50

gcgaatttct tatgatttat gatttttatt attaaataag ttataaaaaa aataagtgta 60

tacaaatttt aaagtgactc ttaggtttta aaacgaaaat tcttattctt gagtaactct 120

ttcctgtagg tcaggttgct ttctcaggta tagcatgagg tcgctcttat tgaccacacc 180

tctaccggca tgccgagcaa atgcctgcaa atcgctcccc atttcaccca attgtagata 240

tgctaactcc agcaatgagt tgatgaatct cggtgtgtat tttatgtcct cagaggacaa 300

cacctgttgt aatcgttctt ccacac 326

<210> SEQ ID NO 51
<211> LENGTH: 374
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: promoter

<400> SEQUENCE: 51

gtgggtgtat tggattatag gaagccacgc gctcaacctg gaattacagg aagctggtaa 60

ttttttgggt ttgcaatcat caccatctgc acgttgttat aatgtcccgt gtctatatat 120

atccattgac ggtattctat ttttttgcta ttgaaatgag cgtttttttgt tactacaatt 180

ggttttacag acggaatttt ccctatttgt ttcgtcccat ttttcctttt ctcattgttc 240

tcatatctta aaaaggtcct ttcttcataa tcaatgcttt cttttactta atattttact 300

tgcattcagt gaattttaat acatattcct ctagtcttgc aaaatcgatt tagaatcaag 360

ataccagcct aaaa 374

<210> SEQ ID NO 52
<211> LENGTH: 390

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: promoter

<400> SEQUENCE: 52

ctcgtaggaa caatttcggg cccctgcgtg ttcttctgag gttcatcttt tacatttgct 60

tctgctggat aattttcaga ggcaacaagg aaaaattaga tggcaaaaag tcgtctttca 120

aggaaaaatc cccaccatct ttcgagatcc cctgtaactt attggcaact gaaagaatga 180

aaaggaggaa aatacaaaat atactagaac tgaaaaaaaa aaagtataaa tagagacgat 240

atatgccaat acttcacaat gttcgaatct attcttcatt tgcagctatt gtaaaataat 300

aaaacatcaa gaacaaacaa gctcaacttg tcttttctaa gaacaaagaa taaacacaaa 360

aacaaaaagt ttttttaatt ttaatcaaaa 390

<210> SEQ ID NO 53
<211> LENGTH: 302
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: terminator

<400> SEQUENCE: 53

acaaatcgct cttaaatata tacctaaaga acattaaagc tatattataa gcaaagatac 60

gtaaattttg cttatattat tatacacata tcatatttct atatttttaa gatttggtta 120

tataatgtac gtaatgcaaa ggaaataaat tttatacatt attgaacagc gtccaagtaa 180

ctacattatg tgcactaata gtttagcgtc gtgaagactt tattgtgtcg cgaaaagtaa 240

aaattttaaa aattagagca ccttgaactt gcgaaaaagg ttctcatcaa ctgtttaaaa 300

gg 302

1. A eukaryotic cell capable of expressing the following nucleotide sequences, wherein the expression of these nucleotide sequences confers on the cell the ability to use L-arabinose and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product:

(a) a nucleotide sequence encoding an arabinose isomerase (araA), wherein said nucleotide sequence is selected from the group consisting of:

i. nucleotide sequences encoding an araA, said araA comprising an amino acid sequence that has at least 55% sequence identity with the amino acid sequence of SEQ ID NO:1,

ii. nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:2,

iii. nucleotide sequences the complementary strand of which hybridizes to a nucleic add molecule of sequence of (i) or (ii);

iv, nucleotide sequences the sequences of which differs from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic; code,

(b) a nucleotide sequence encoding a L-ribulokinase (araB), wherein said nucleotide sequence is selected from the group consisting of:

i. nucleotide sequences encoding an araB, said araB comprising an amino acid sequence that has at least 20% sequence identity with the amino acid sequence of SEQ ID NO:3,

ii. nucleotide sequences comprising a nucleotide sequence that has at least 50% sequence identity with the nucleotide sequence of SEQ ID NO:4,

iii. nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

iv. nucleotide sequences the sequences of which differs from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code,

(c) a nucleotide sequence encoding an L-ribulose-5-P-4-epimerase (araD), wherein said nucleotide sequence is selected from the group consisting of:

i. nucleotide sequences encoding an araD, said araD comprising an amino acid sequence that has at least 60% sequence identity with the amino acid sequence of SEQ ID NO:5,

ii. nucleotide sequences comprising a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO:6,

iii. nucleotide sequences the complementary strand of which hybridizes to a nucleic acid molecule of sequence of (i) or (ii);

iv. nucleotide sequences the sequences of which differs from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code.

2. A cell according to claim **1**, wherein one, two or three of the araA, araB and araD nucleotide sequences originate from a *Lactobacillus* genus, preferably a *Lactobacillus plantarum* species.

3. A cell according to claim **1**, wherein the cell is a yeast cell, preferably belonging to one of the genera: *Saccharomyces, Kluyveromyces, Candida, Pichia, Schizosaccharomyces, Hansenula, Kloeckera, Schwanniomyces* or *Yarrowia*.

4. A cell according to claim **3**, wherein the yeast cell belongs to one of the species: *S. cerevisiae, S. bulderi, S. barnetti, S. exiguus, S. uvarum, S. diastaticus, K. lactis, K. marxianus* or *K. fragilis*.

5. A cell according to claim **1**, wherein the nucleotide sequences encoding the araA, araB and/or araD are operably linked to a promoter that causes sufficient expression of the corresponding nucleotide sequences in the cell to confer to the cell the ability to use L-arabinose and/or to convert L-arabinose into L-ribulose, and/or xylulose 5-phosphate and/or into a desired fermentation product.

6. A cell according to claim **1**, wherein the cell exhibits the ability to directly isomerise xylose into xylulose.

7. A cell according to claim **6**, wherein the cell comprises a genetic modification that increases the flux of the pentose phosphate pathway.

8. A cell according to claim **6**, wherein the genetic modification comprises overexpression of at least one gene of the non-oxidative part of the pentose phosphate pathway.

9. A cell according to claim **8**, wherein the gene is selected from the group consisting of the genes encoding ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transketolase and transaldolase.

10. A cell according to claim **8**, wherein the genetic modification comprises overexpression of at least the genes coding for a transketolase and a transaldolase.

11. A cell according to claim **1**, wherein the cell further comprises a genetic modification that increases the specific xylulose kinase activity.

12. A cell according to claim **11**, wherein the genetic modification comprises overexpression of a gene encoding a xylulose kinase.

13. A cell according to claim **8**, wherein the gene that is overexpressed is endogenous to the cell.

14. A cell according to claim **5**, wherein the cell comprises a genetic modification that reduces unspecific aldose reductase activity in the cell.

15. A cell according to claim **14**, wherein the genetic modification reduces the expression of, or inactivates a gene encoding an unspecific aldose reductase.

16. A cell according to claim **15**, wherein the gene is inactivated by deletion of at least part of the gene or by disruption of the gene.

17. A cell according to claim **14**, wherein the expression of each gene in the cell that encodes an unspecific aldose reductase is reduced or inactivated.

18. A cell according to claim **1**, wherein the fermentation product is selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin.

19. A nucleic acid construct comprising a nucleic acid sequence encoding an araA, a nucleic acid sequence encoding an araB and/or a nucleic acid sequence encoding an araD all as defined in claim **1**.

20. A process for producing a fermentation product selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam, antibiotic and a cephalosporin, whereby the process comprises:

(a) fermenting a medium containing a source of arabinose and optionally xylose with a modified cell as defined in claim **1**, whereby the cell ferments arabinose and optionally xylose to the fermentation product; and optionally,

(b) recovering the fermentation product.

21. A process for producing a fermentation product selected from the group consisting of ethanol, lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, malic acid, fumaric acid, an amino acid, 1,3-propane-diol, ethylene, glycerol, butanol, a β-lactam antibiotic and a cephalosporin, wherein the process comprises:

(a) fermenting a medium containing at least a source of L-arabinose and a source of xylose with a cell as defined in claim **1** and a cell able to use xylose and/or exhibiting the ability to directly isomerise xylose into xylulose, whereby each cell ferments L-arabinose and/or xylose to the fermentation product; and optionally,

(b) recovering the fermentation product.

22. A process according to claim **20**, wherein the medium also contains a source of glucose.

23. A process according to claim **20**, wherein the fermentation product is ethanol.

24. A process according to claim **23**, wherein the volumetric ethanol productivity is at least 0.5 g ethanol per litre per hour.

25. A process according to claim **23**, wherein the ethanol yield is at least 30%.

26. A process according to claim **20**, wherein the process is anaerobic.

27. A process according to claim **20**, wherein the process is aerobic, preferably performed under oxygen limited conditions.

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