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(54) **COMPETITIVE GROWTH AND/OR PRODUCTION ADVANTAGE FOR BUTANOLOGEN MICROORGANISM**

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(51) **Int. Cl.**
C12P 7/16 (2006.01)
C12N 1/00 (2006.01)
C12P 7/10 (2006.01)

(52) **U.S. Cl.**
CPC *C12P 7/16* (2013.01); *C12P 7/10* (2013.01); *Y02E 50/10* (2013.01); *Y02E 50/16* (2013.01)

(58) **Field of Classification Search**
CPC C12P 7/16; C12P 7/10; Y02E 50/16; Y02E 50/10
USPC 435/160, 254.11
See application file for complete search history.

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(57) **ABSTRACT**

Provided herein are recombinant yeast host cells and methods for their use for production of fermentation products. Host cells provided herein comprise a pyruvate-utilizing pathway and a competitive growth advantage over other microorganisms in solution.

10 Claims, 19 Drawing Sheets

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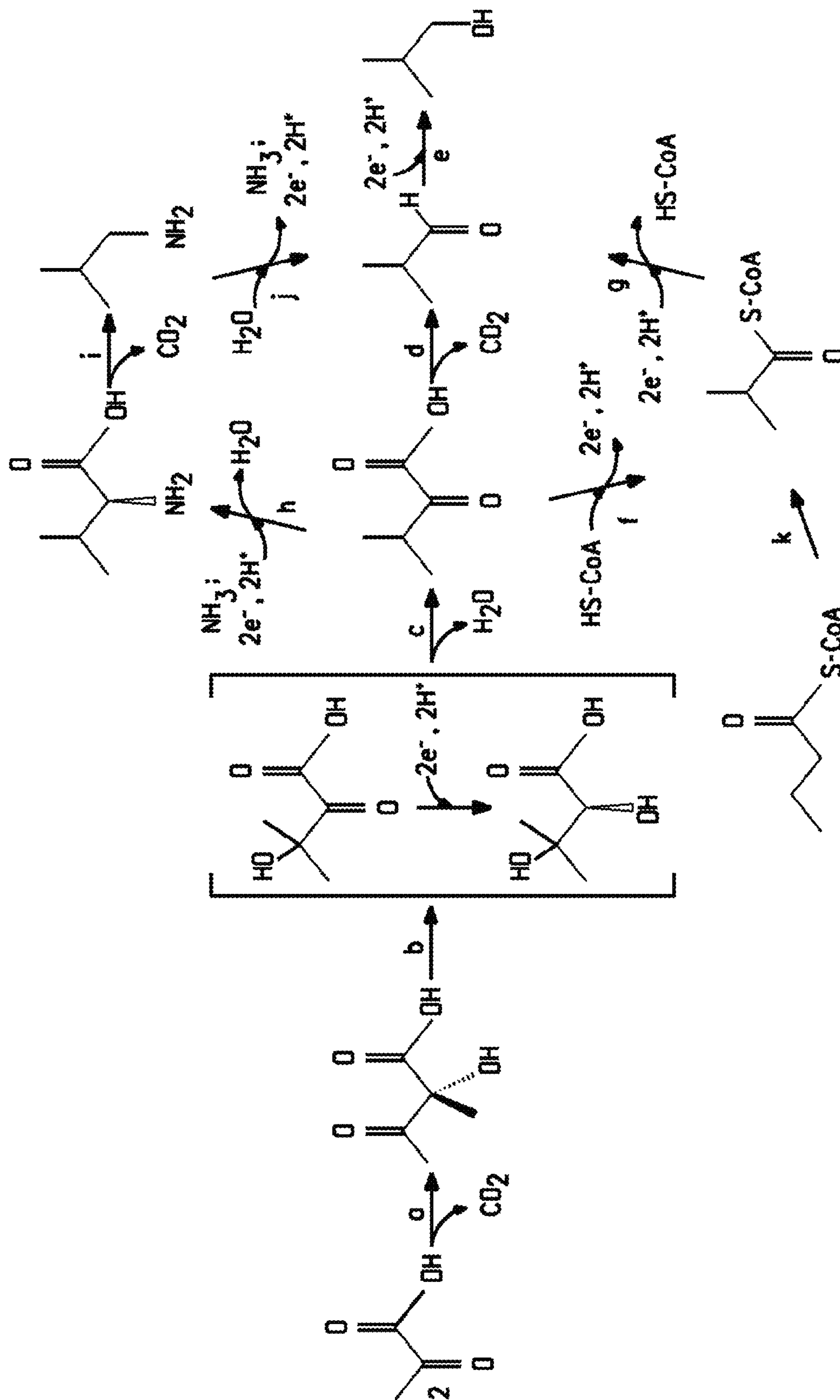


FIG. 1

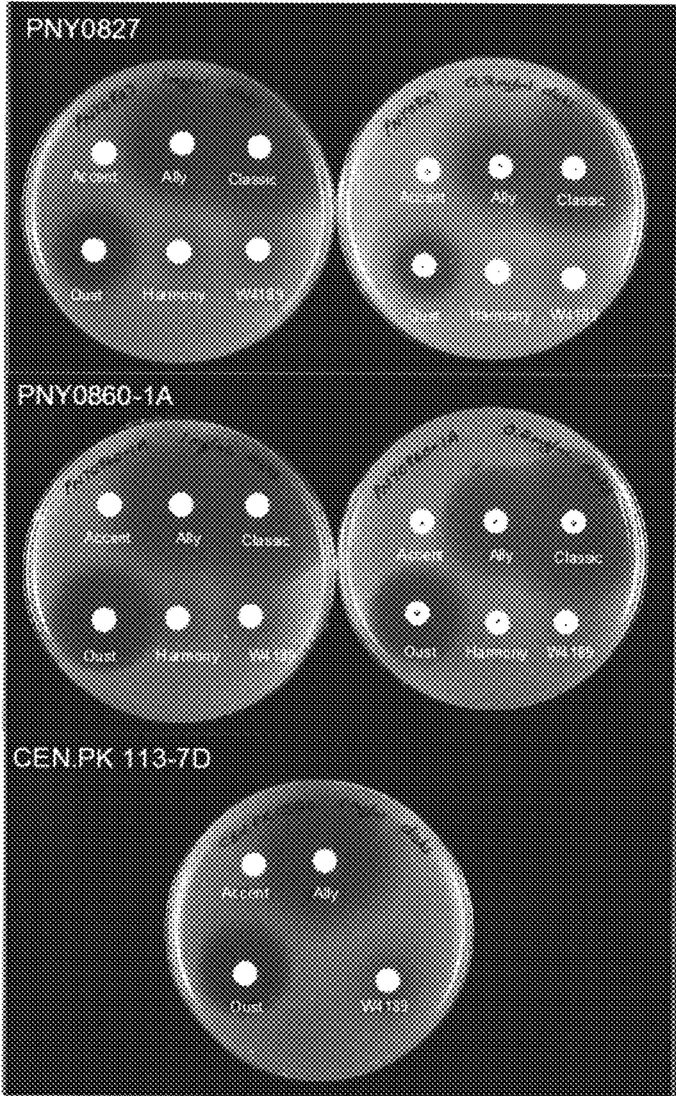


FIG. 2

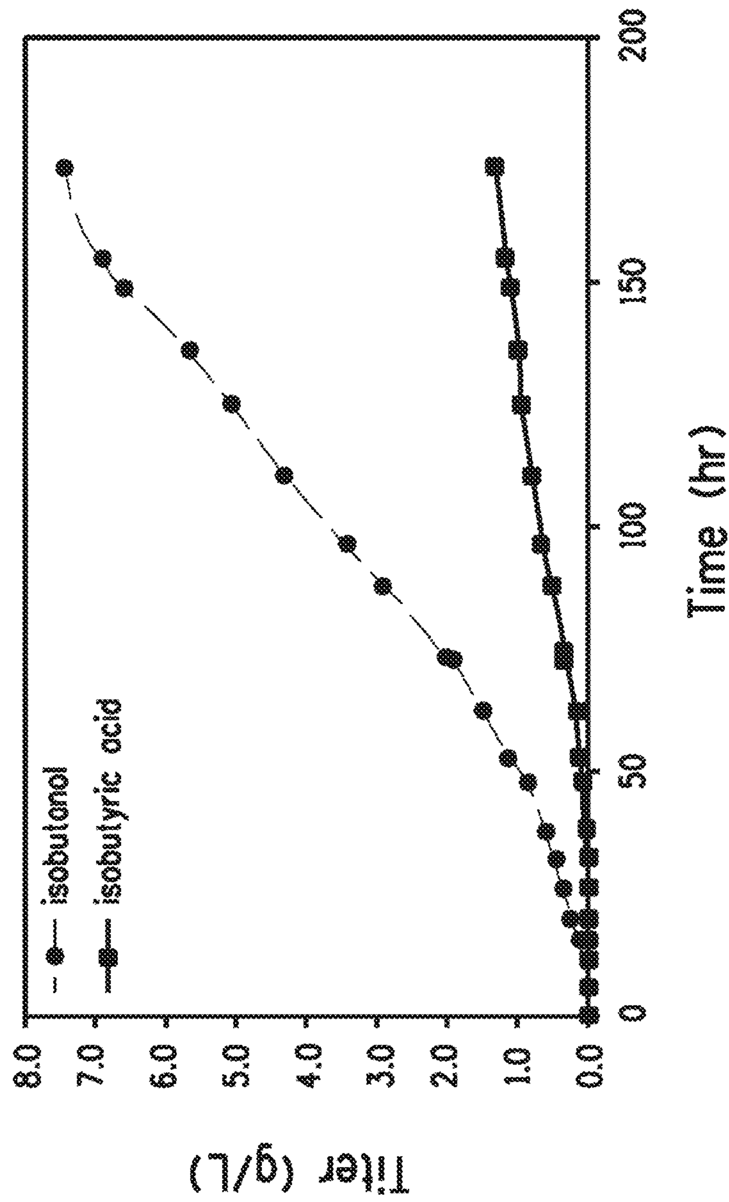


FIG. 3

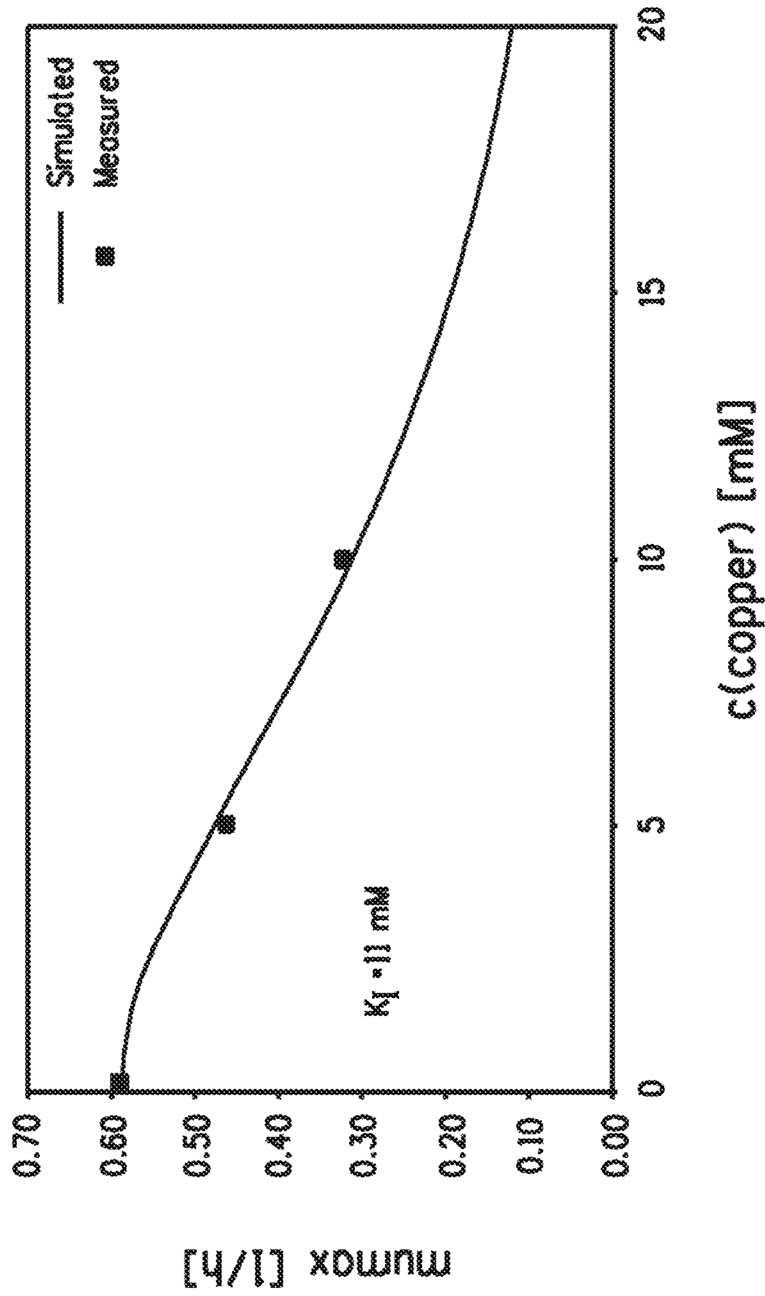


FIG. 4

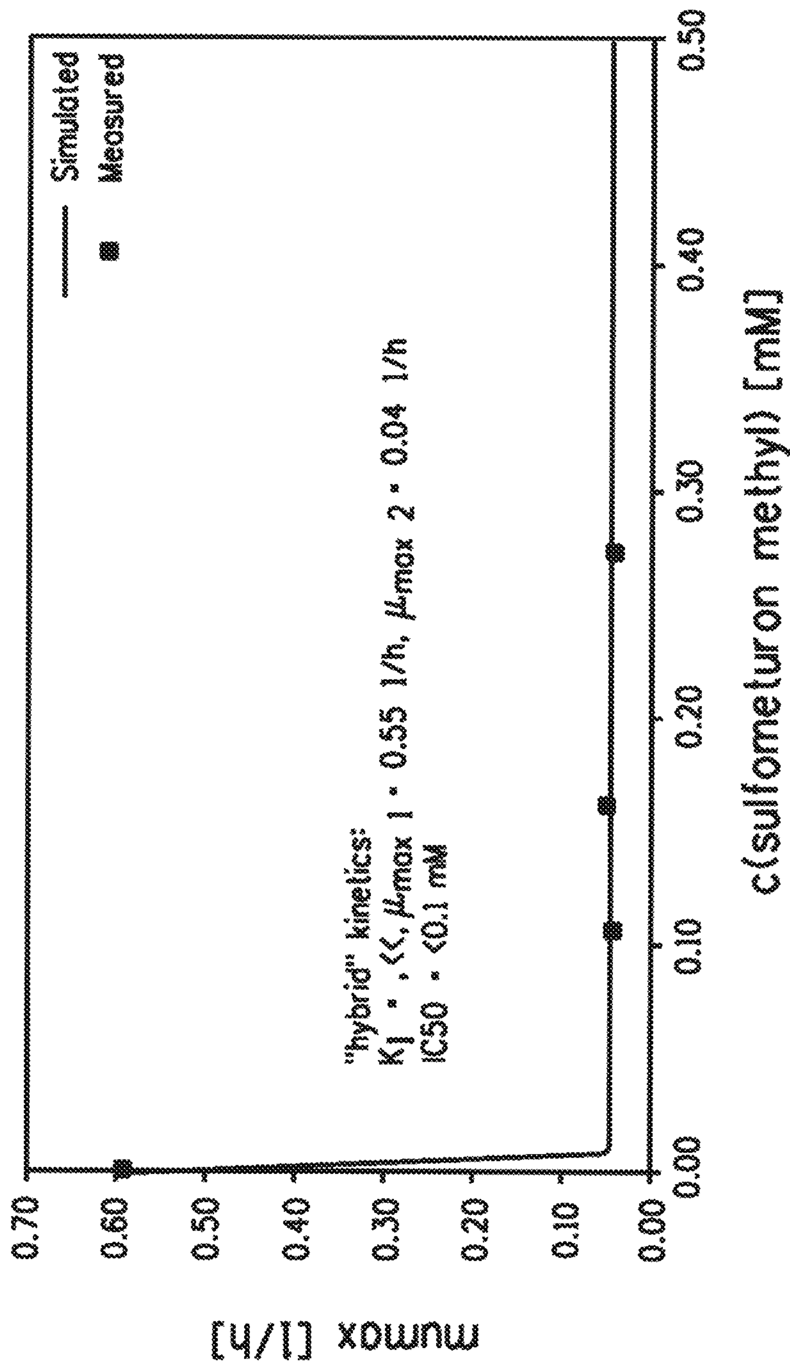


FIG. 5

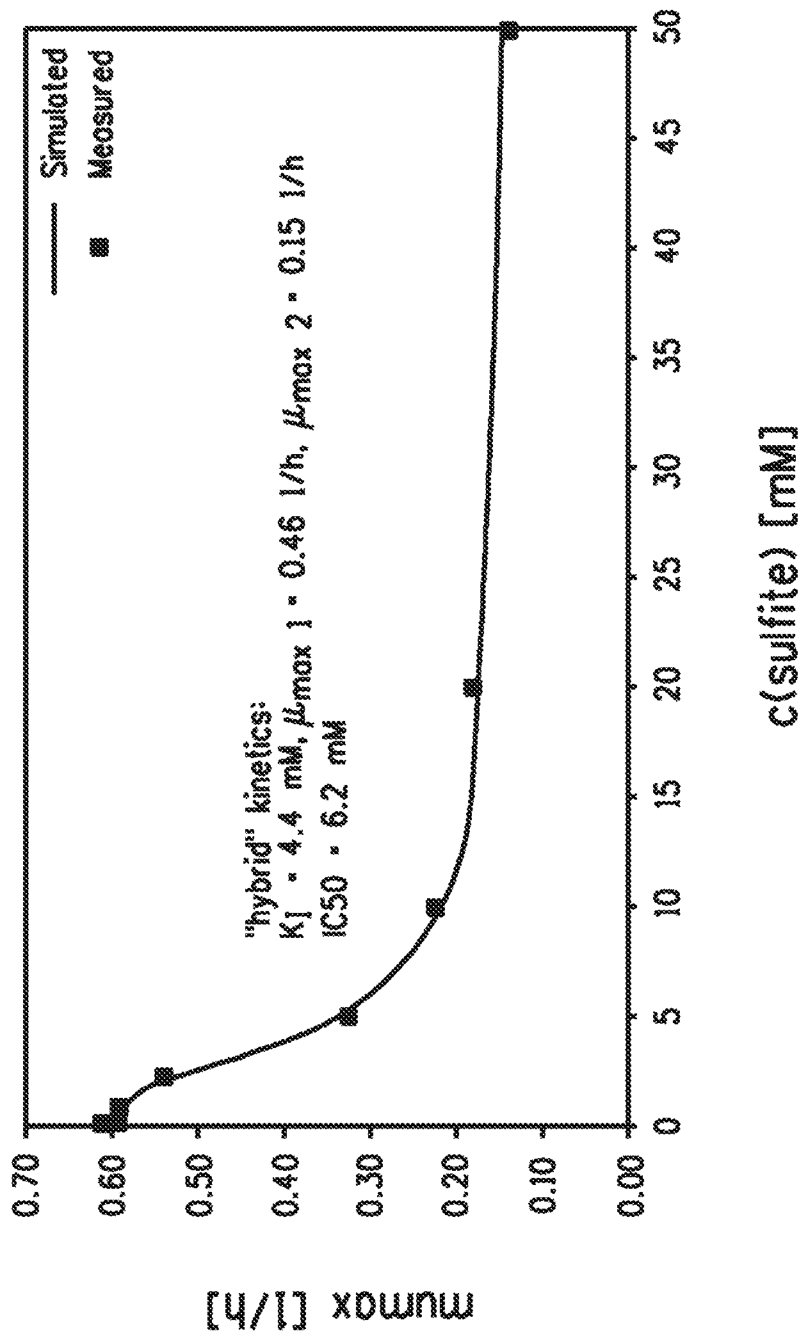
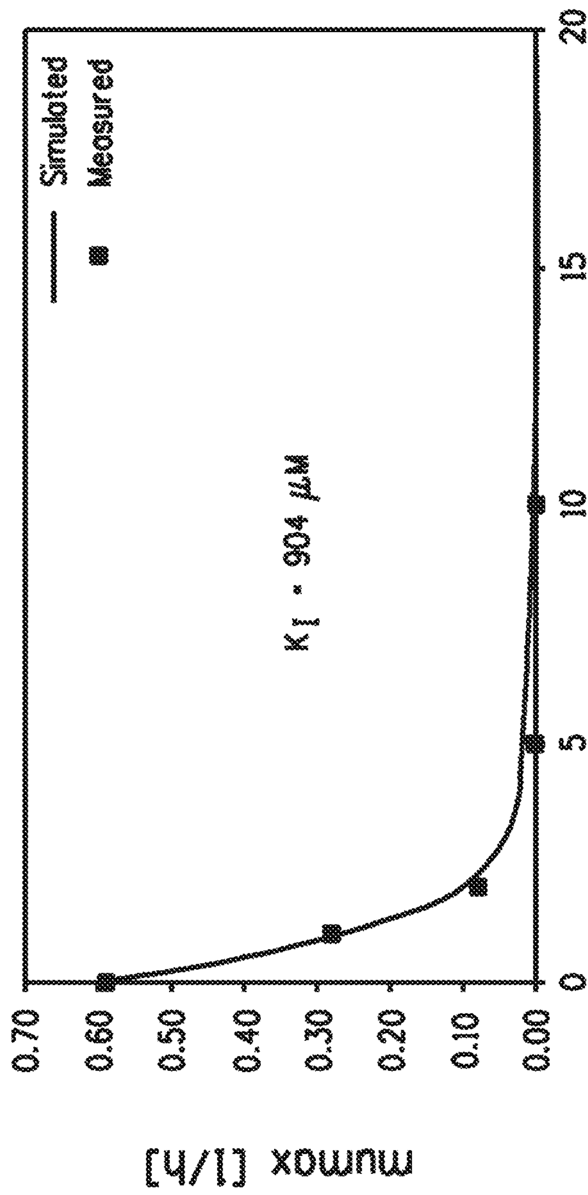


FIG. 6



c(formaldehyde) [mM]

FIG. 7

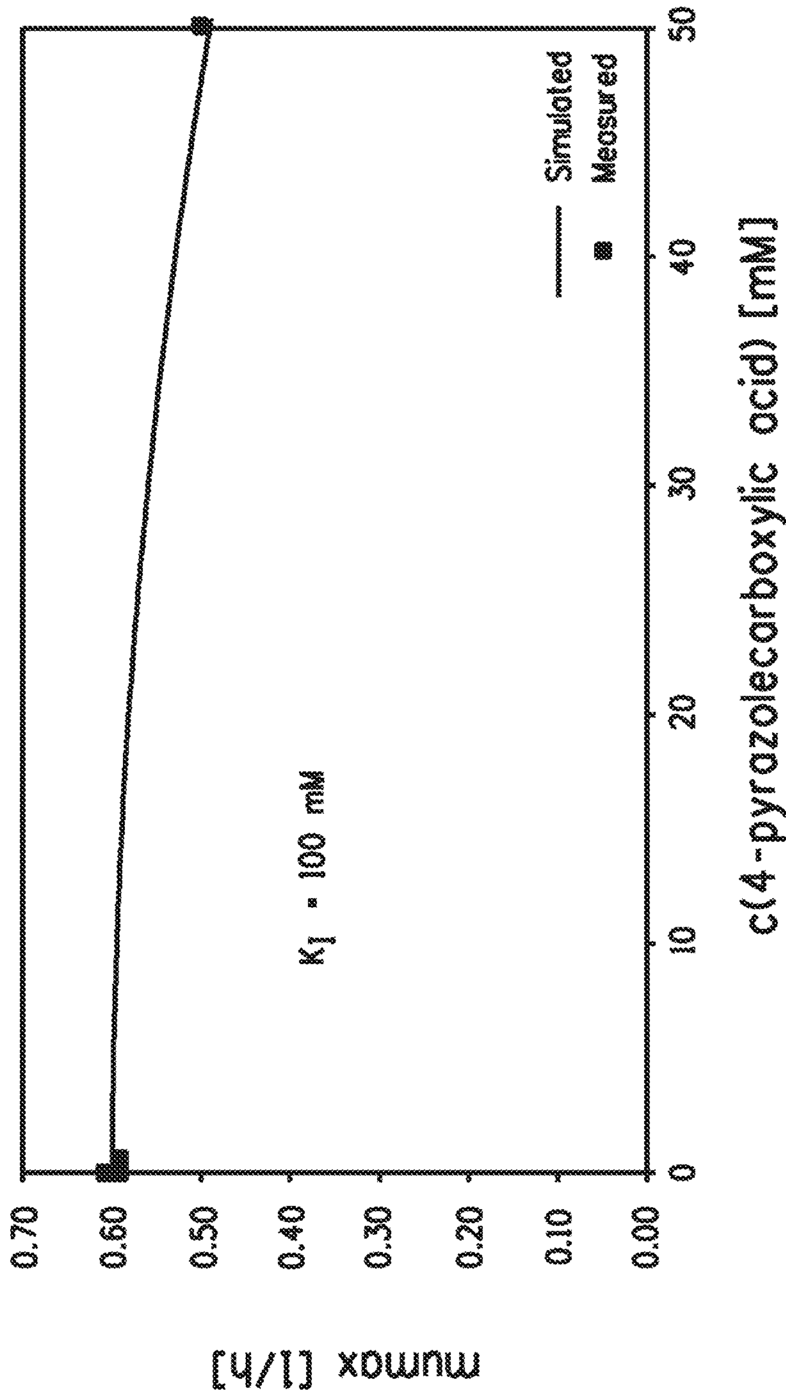


FIG. 8

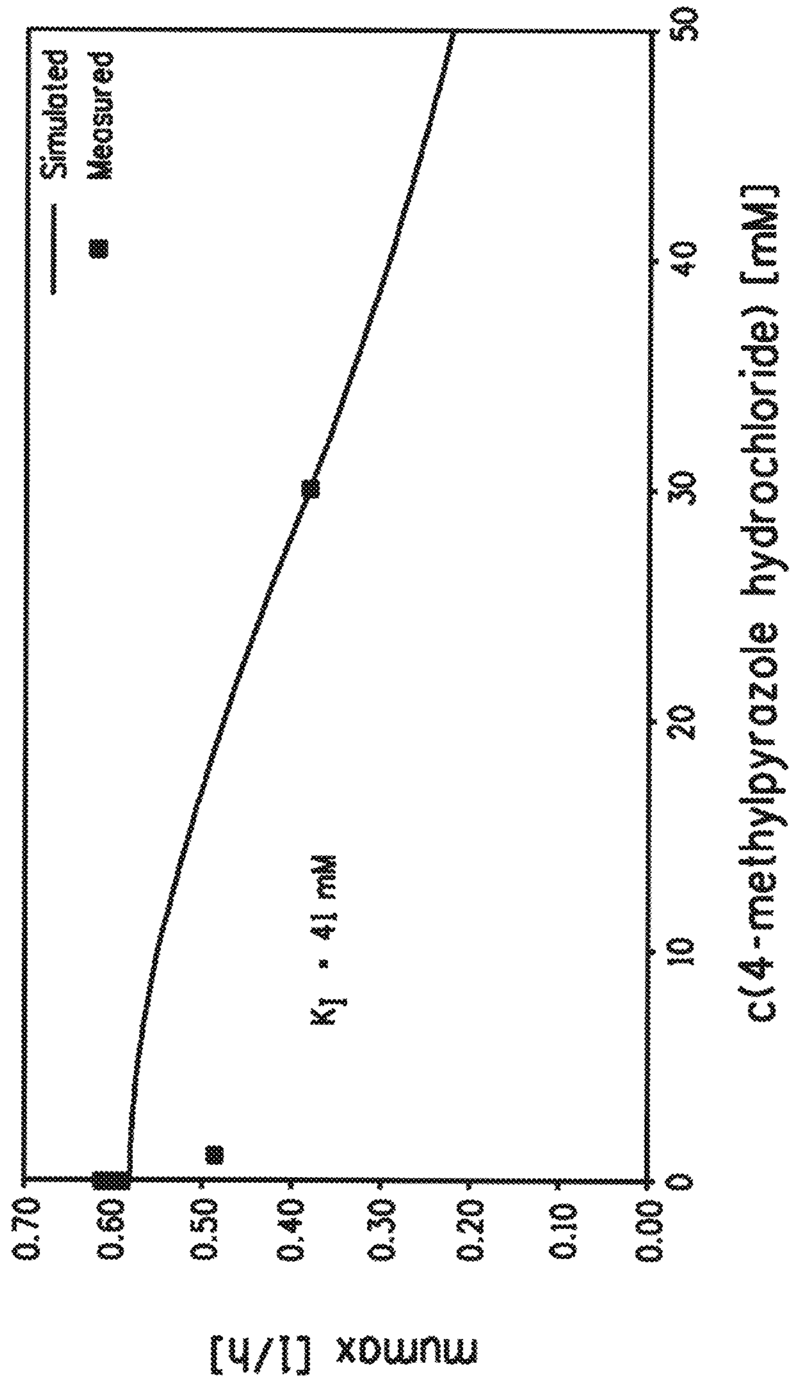


FIG. 9

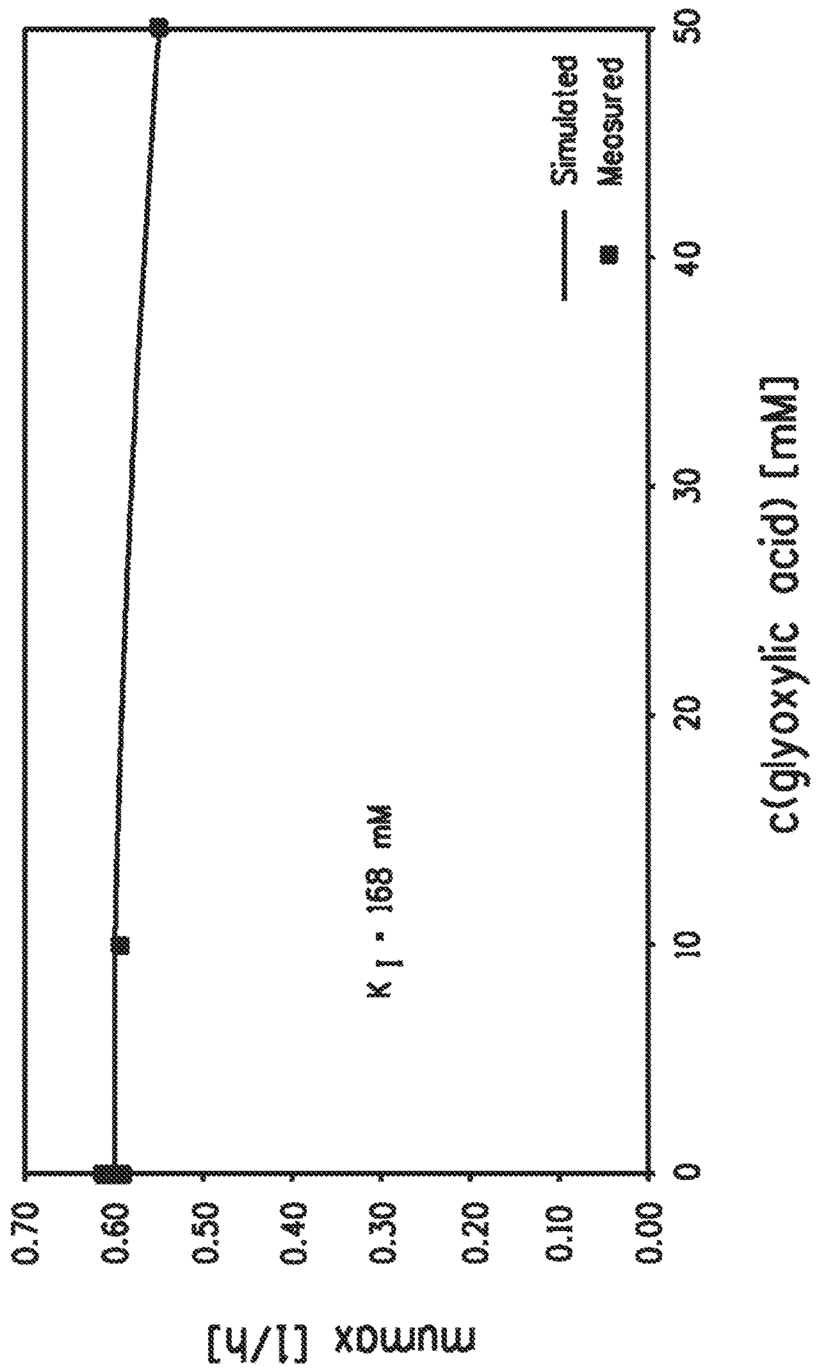


FIG. 10

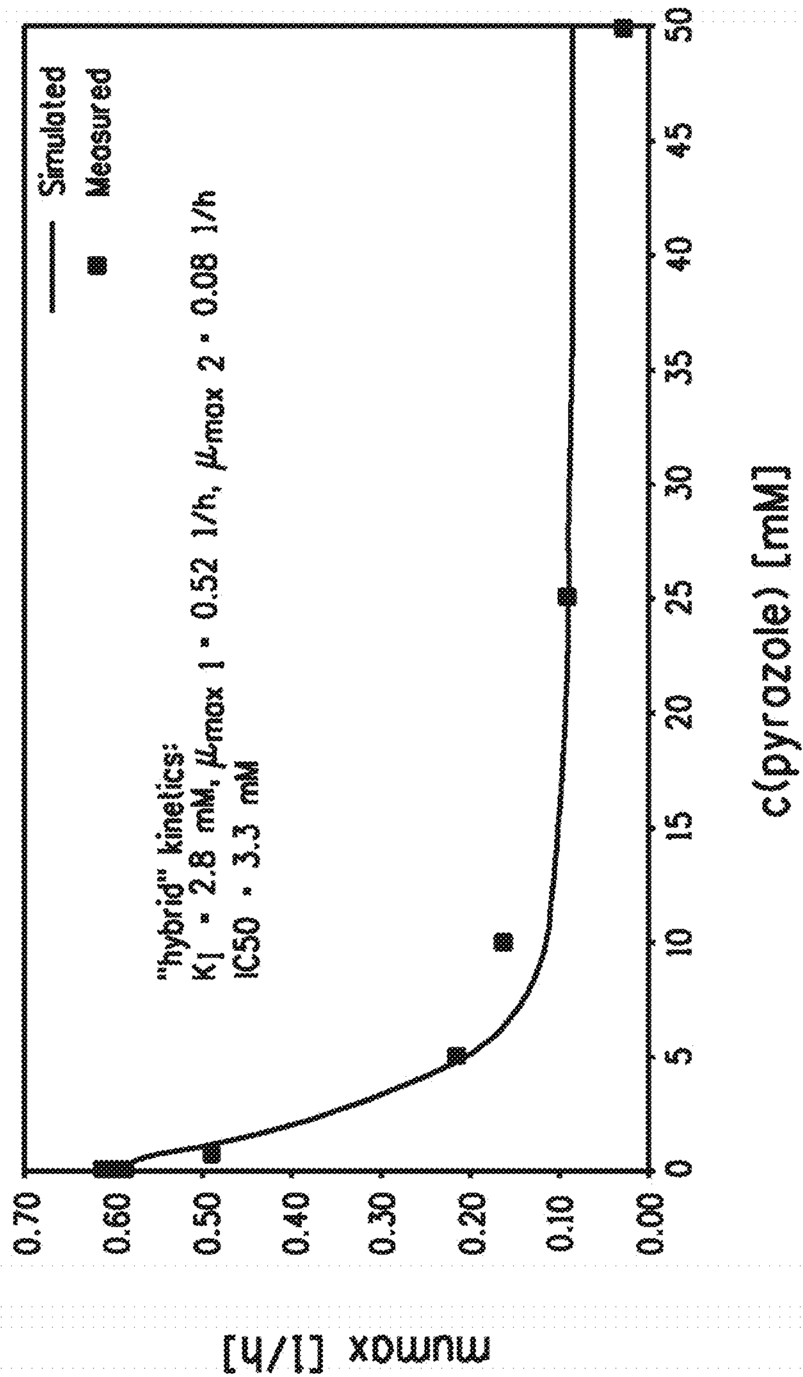


FIG. 11

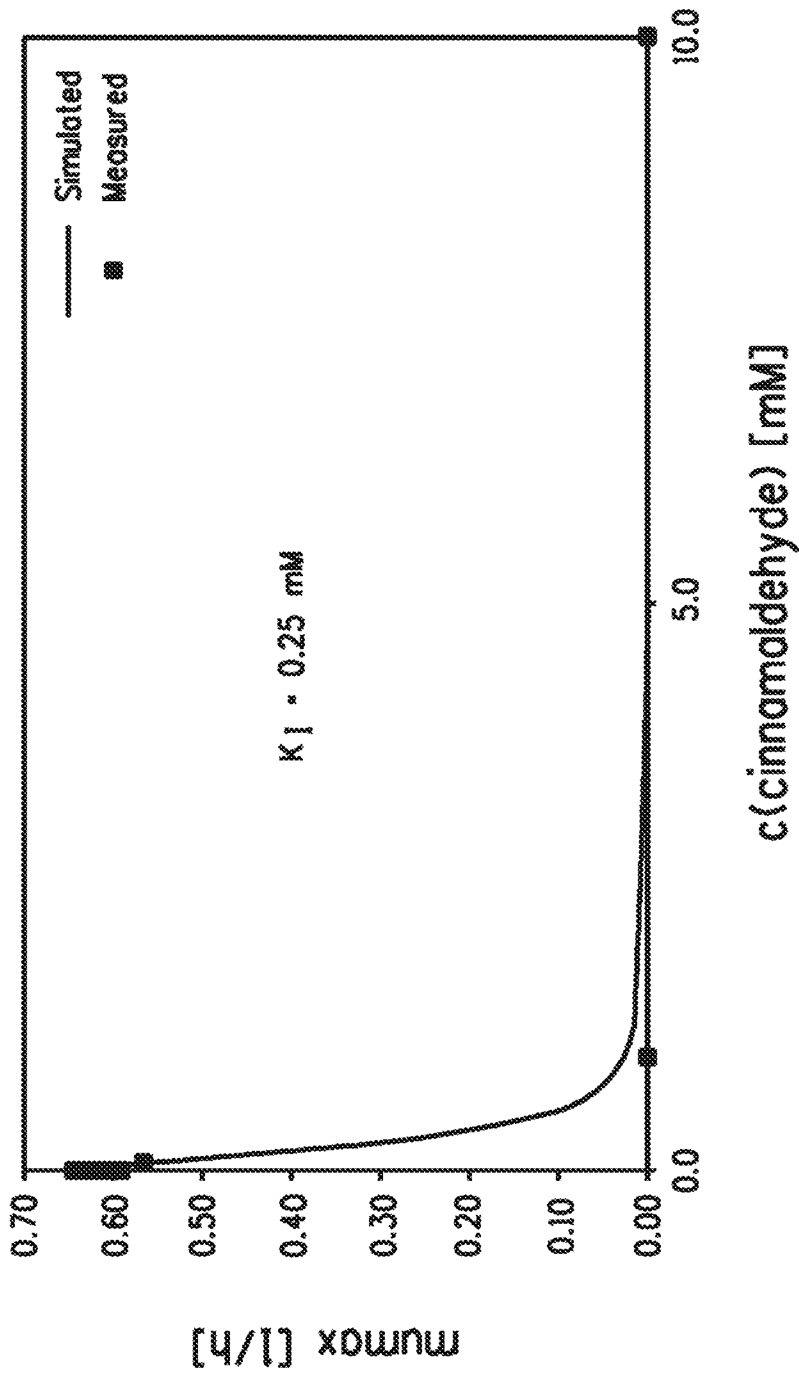


FIG. 12

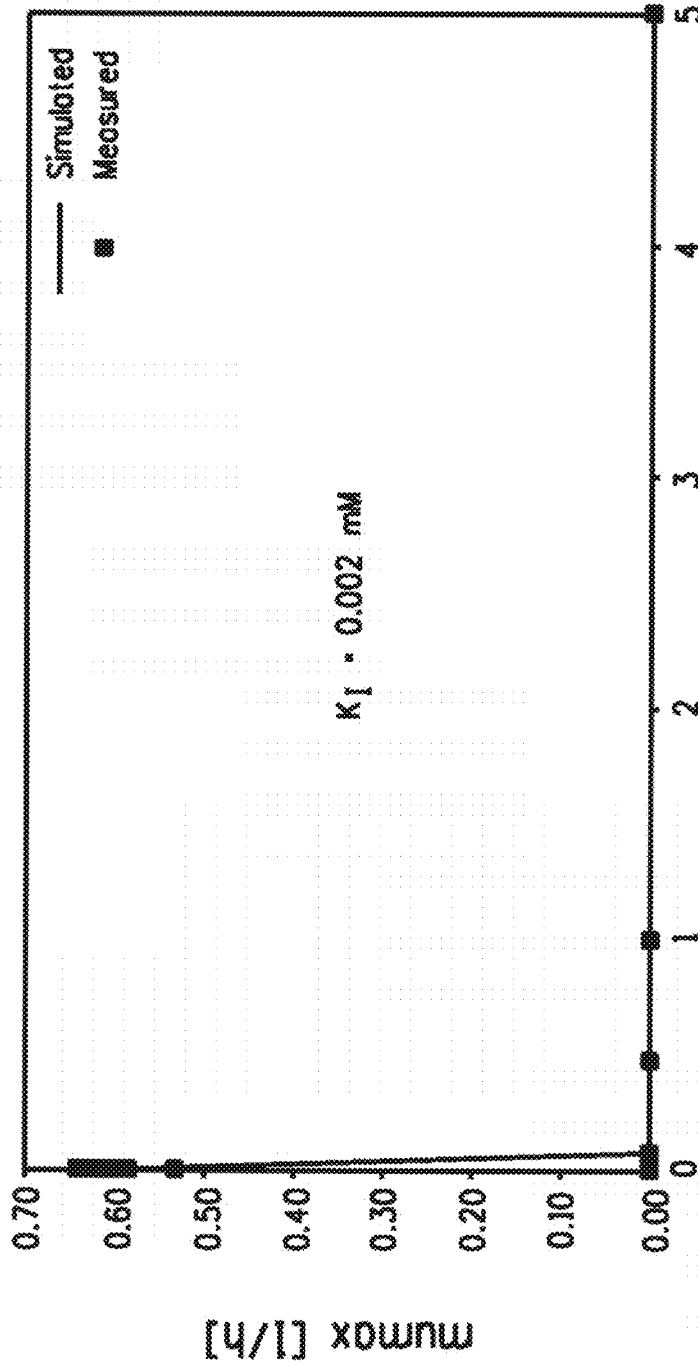


FIG. 13

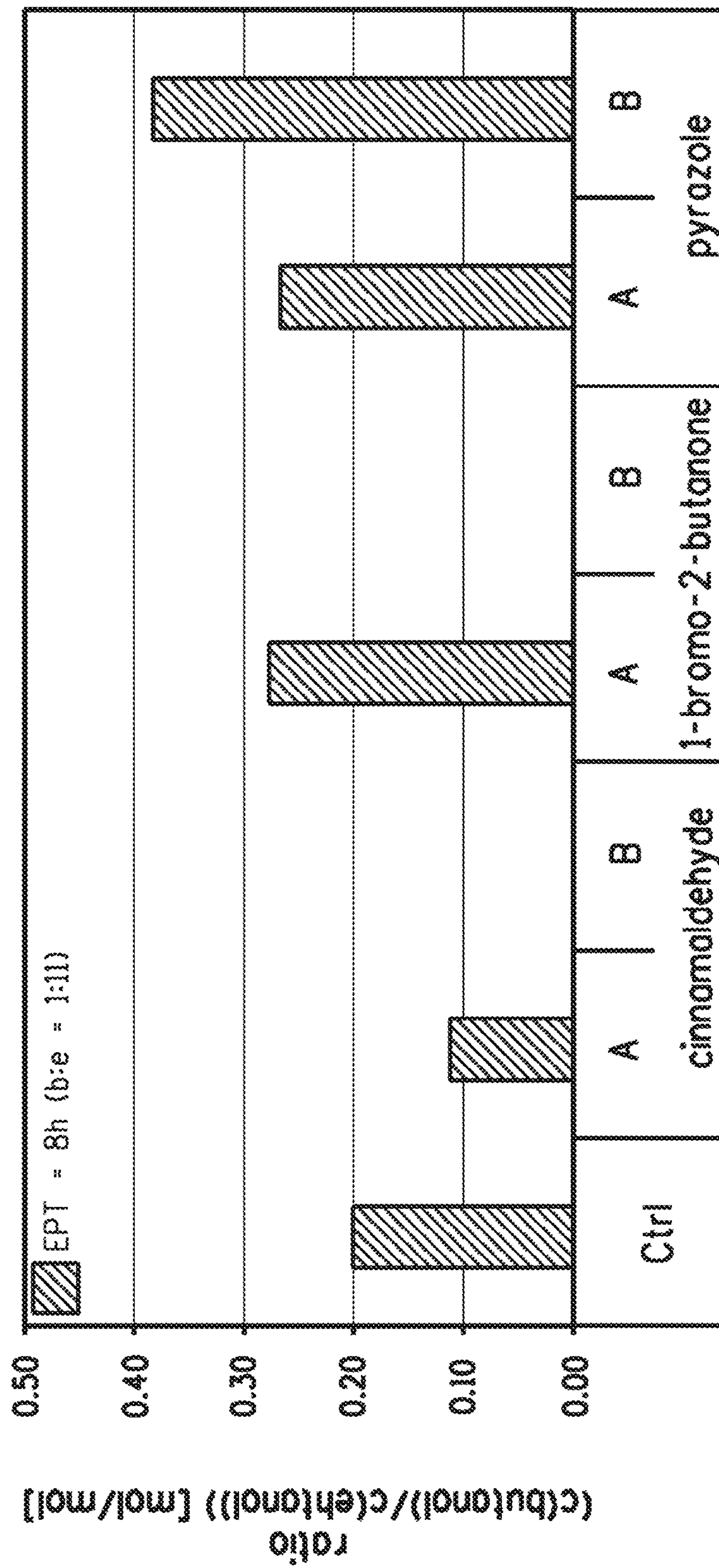


FIG. 14

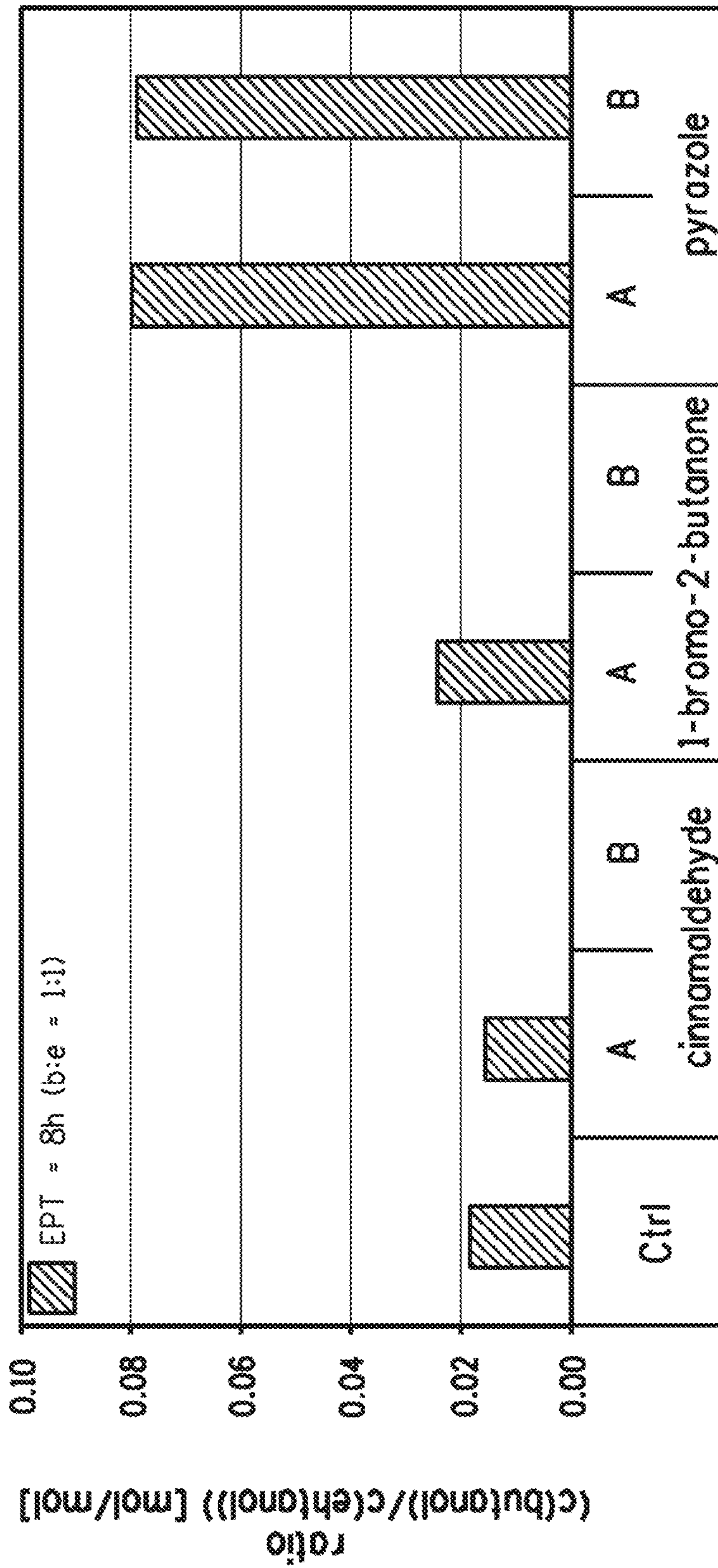


FIG. 15

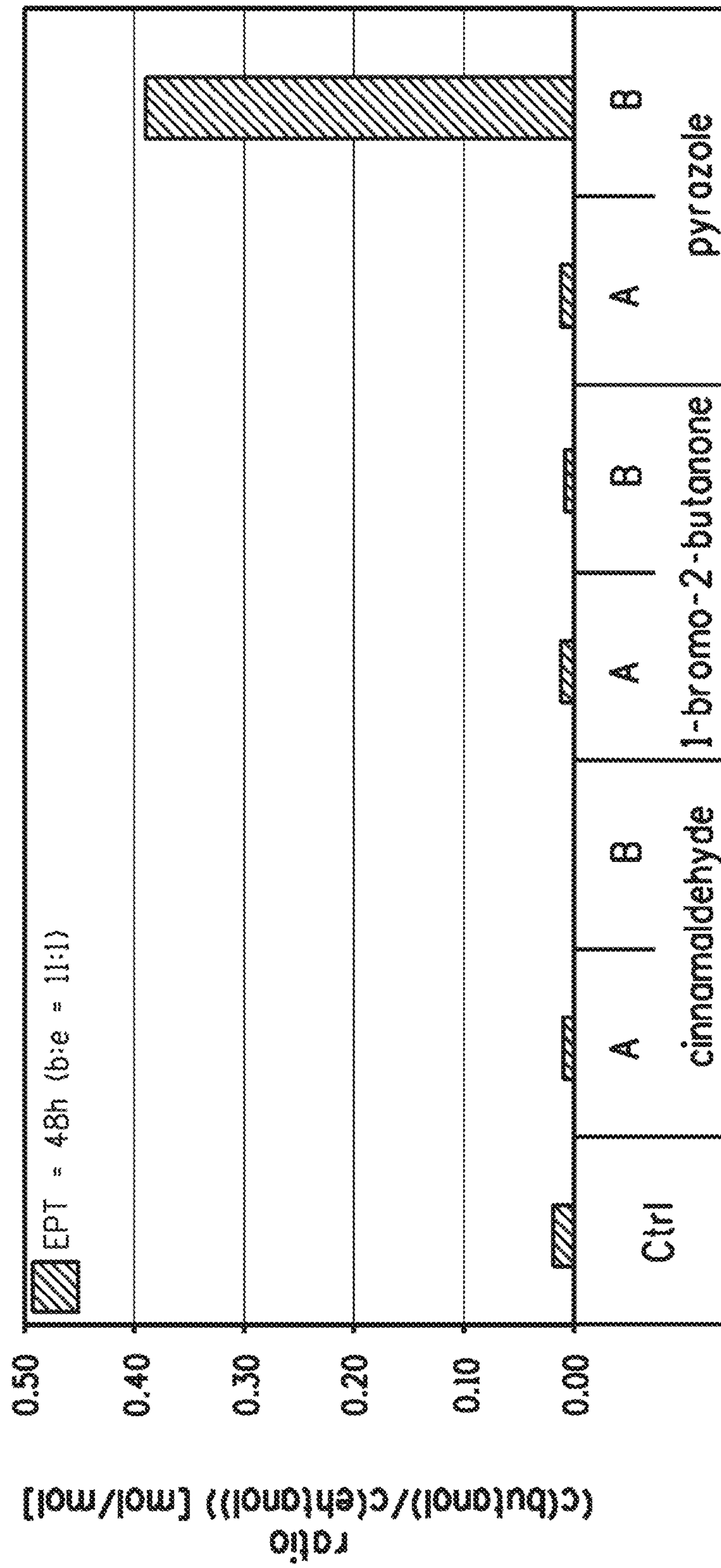


FIG. 16

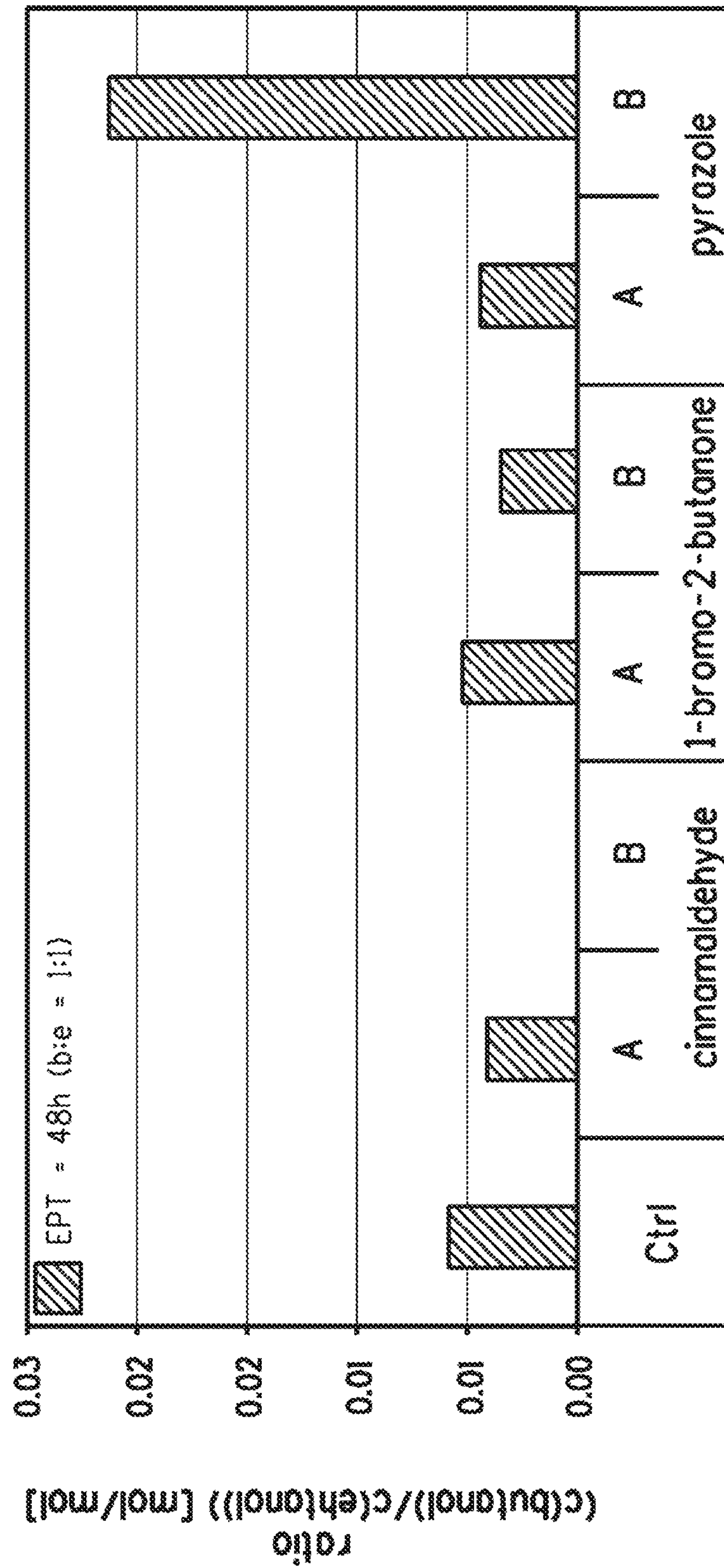


FIG. 17

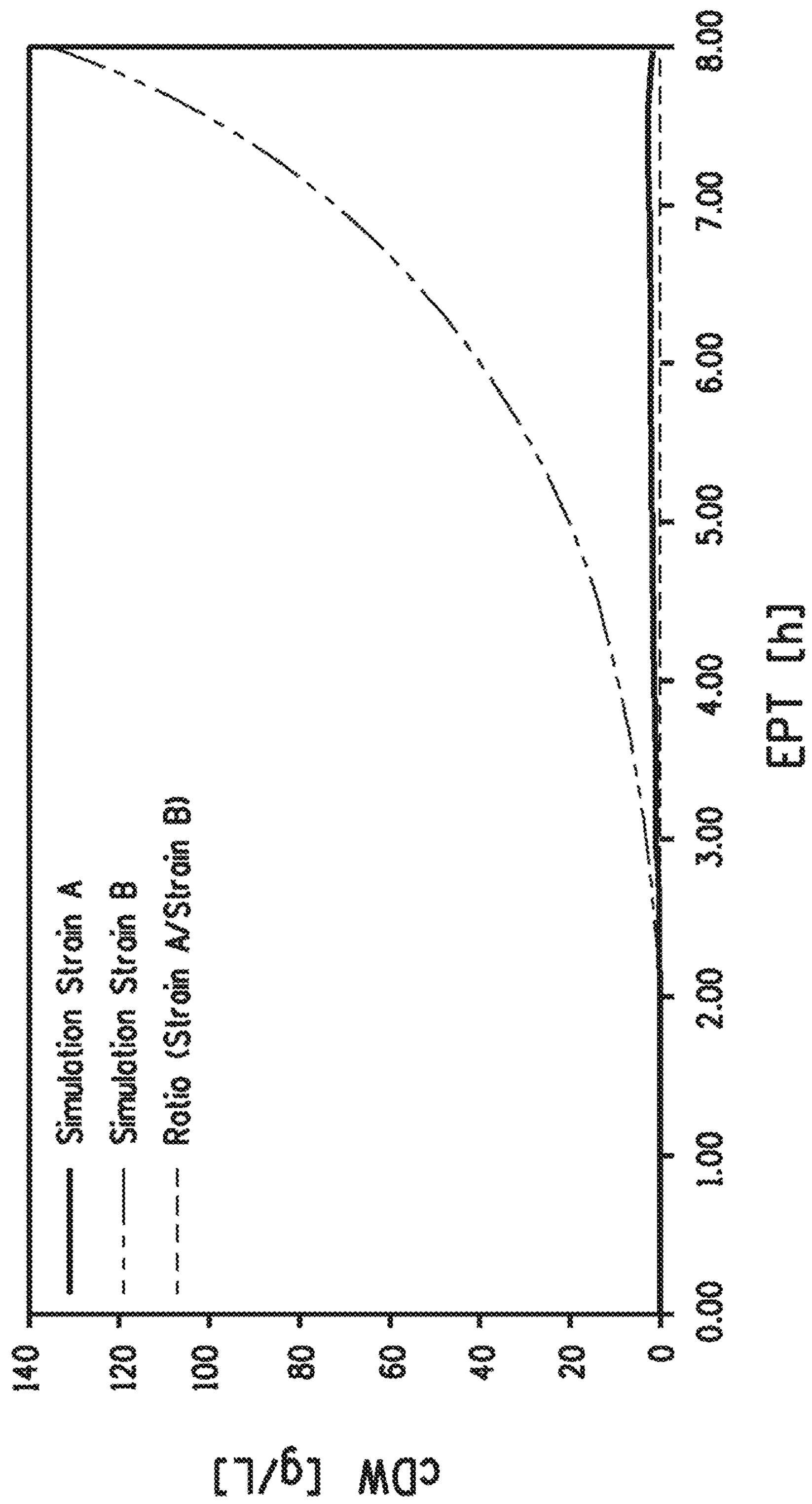


FIG. 18

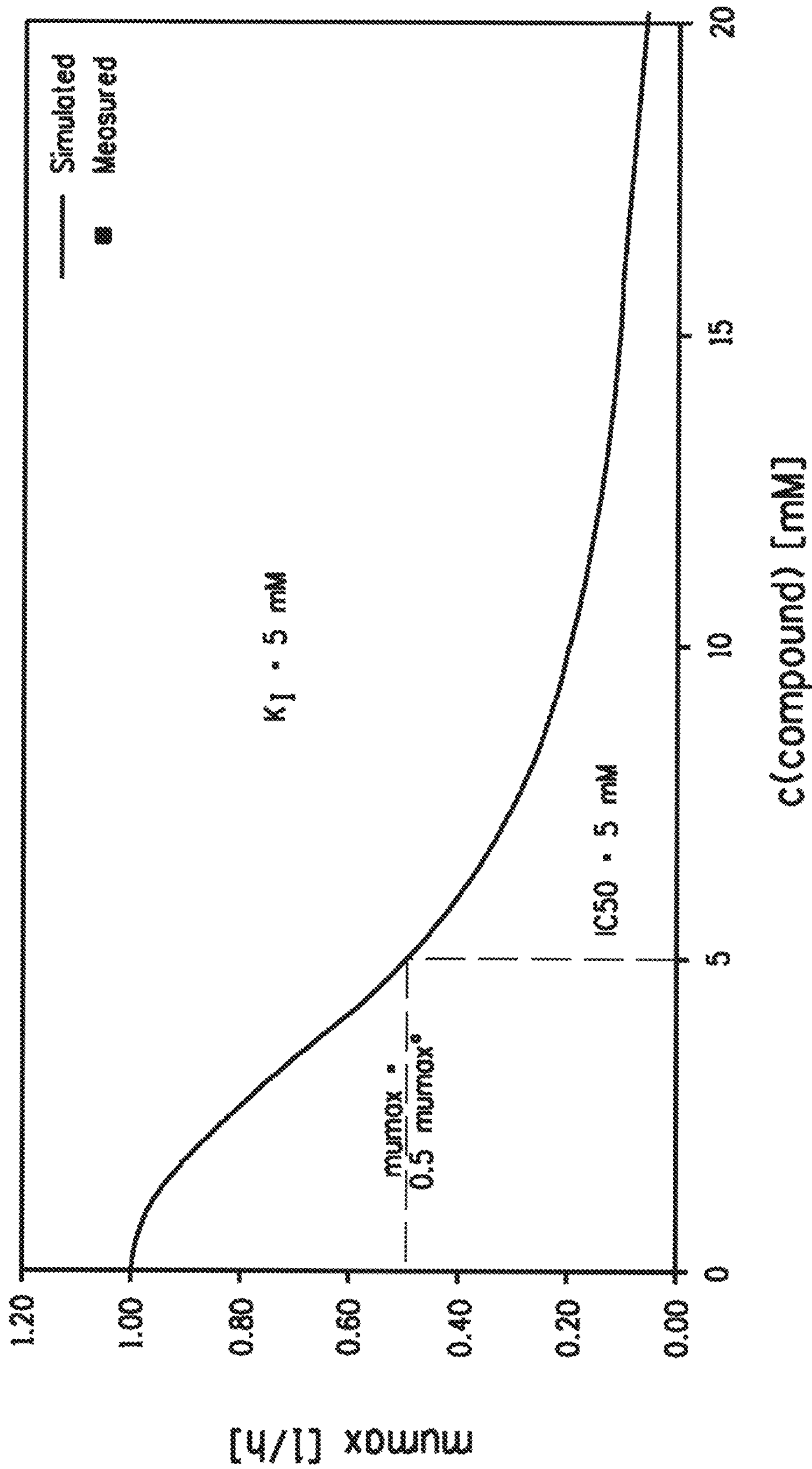


FIG. 19

COMPETITIVE GROWTH AND/OR PRODUCTION ADVANTAGE FOR BUTANOLOGEN MICROORGANISM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application No. 61/801,239, filed Mar. 15, 2013, which is hereby incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

The content of the electronically submitted sequence listing (Name: 20140314_CL5075USNP_SequenceListing.txt; Size: 498,298 bytes; and Date of Creation: Mar. 14, 2014) is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates to the fields of industrial microbiology and alcohol production. The invention also relates to the development of an industrial microorganism capable of producing fermentation products via an engineered pyruvate-utilizing pathway in the microorganism. The invention also relates to the development and use of a butanologen. The invention also relates to the use of inhibitors, antibiotics, and mixtures thereof to give the butanologen a competitive growth and/or production advantage over other organisms in culture in order to increase the yield of fermentation products.

BACKGROUND OF THE INVENTION

Butanol is an important industrial chemical, useful as a fuel additive, as a feedstock chemical in the plastics industry, and as a food grade extractant in the food and flavor industry. Each year 10 to 12 billion pounds of butanol are produced by petrochemical means.

Methods for the chemical synthesis of the butanol isomer, isobutanol, are known, such as oxo synthesis, catalytic hydrogenation of carbon monoxide (Ullmann's Encyclopedia of Industrial Chemistry, 6th edition, 2003, Wiley-VCH Verlag GmbH and Co., Weinheim, Germany, Vol. 5, pp. 716-719) and Guerbet condensation of methanol with n-propanol (Carlini et al., *J. Molec. Catal. A. Chem.* 220:215-220, 2004). These processes use starting materials derived from petrochemicals. The production of isobutanol from plant-derived raw materials could minimize the use of fossil fuels and would represent an advance in the art. Microorganisms capable of fermentative production of isobutanol have been described (for example, in U.S. Pat. Nos. 7,851,188 and 7,993,889).

Isobutanol is produced biologically as a by-product of yeast fermentation. It is a component of "fusel oil" that forms as a result of the incomplete metabolism of amino acids by this group of fungi. Isobutanol may be produced from catabolism of L-valine. After the amine group of L-valine is harvested as a nitrogen source, the resulting α-keto acid is decarboxylated and reduced to isobutanol by enzymes of the so-called Ehrlich pathway (Dickinson et al., *J. Biol. Chem.* 273:25752-25756, 1998). Microorganisms expressing engineered biosynthetic pathways for producing butanol isomers, including isobutanol, are also described (see U.S. Pat. Nos. 7,851,188 and 7,993,889, which are incorporated herein by reference).

SUMMARY OF THE INVENTION

In some embodiments, the invention is directed to a method for production of a fermentation product in a fermentation process comprising contacting a fermentation mix comprising a recombinant production microorganism which comprises a pyruvate-utilizing pathway with at least one compound which preferentially inhibits at least one contaminant yeast microorganism.

In some embodiments, the specific growth rate of the at least one contaminant microorganism is reduced more than the specific growth rate of the recombinant production microorganism.

In some embodiments, production of the fermentation product of the at least one contaminant microorganism is reduced more than production of the fermentation product of the recombinant production microorganism.

In some embodiments, both the production microorganism and the at least one contaminant microorganism are yeast microorganisms. In some embodiments, the contaminant yeast microorganism is *S. cerevisiae.*

In some embodiments, the pyruvate utilizing pathway is a butanol biosynthetic pathway. In some embodiments, the pyruvate utilizing pathway is an isobutanol biosynthetic pathway. In some embodiments, the fermentation product of the at least one contaminant microorganism is ethanol.

In some embodiments, the mechanism of action of the compound that inhibits is heavy metal toxicity, inhibition of amino acid biosynthesis, sulfitolysis, cross-linking, inhibition of ethanol dehydrogenase or inhibition of pyruvate decarboxylase.

In some embodiments, the inhibitor is an inhibitor of an ethanol biosynthesis pathway. In some embodiments, the inhibitor inhibits pyruvate decarboxylase and/or ethanol dehydrogenase. In some embodiments, the inhibitor comprises a member of the XC6H4CH=CHCOCOOH class of inhibitors/substrate analogues, cinnamaldehydes, glyoxalic acid, ketomalonate, regulatory site inhibitors, p chloromercuribenzoic acid, 5,5'-dithiobis(2-nitrobenzoic acid), pyrazole, 4-pyrazolecarboxylic acid, 1-H-pyrazole-1-carboxamidine-HCl, 4-methylpyrazole, 1-bromo-2-butanone, pyrazole-3,5-dicarboxylic acid monohydrate and mixtures thereof. In some embodiments, the inhibitor is selected from the group consisting of fluoroacetate, formaldehyde, sulfite, and mixtures thereof. In some embodiments, the inhibitor is an inhibitor of an amino acid biosynthesis pathway. In some embodiments, the inhibitor is inhibiting at least one enzyme selected from the group consisting of 5-enolpyruvoyl-shikimate-3-phosphate synthetase, α-isopropyl malate synthase, 3-deoxy-D-arabino-heptolusonate-7-phosphate synthase and mixtures thereof. In some embodiments, the inhibitor is selected from the group consisting of imidazolinone, triazolopyrimidine, pyrimidinyl oxybenzoate, sulfonylurea, sulfonylamino carbonyl triazolinone, glyphosate, trifluoroleucine, fluorophenyalanine and mixtures thereof. In some embodiments, the inhibitor is glyphosate. In some embodiments, the inhibitor is selected from a group consisting of nicosulfuron methyl, metsulfuron methyl, chlorimuron ethyl, sulfometuron methyl, chlorsulfuron, thifensulfuron methyl, and mixtures thereof. In some embodiments, the inhibitor is selected from a group consisting of aureobasidin A, bialaphos, cerulenin, chloramphenicol, cyclohexamide, geneticin, hygromycin B, metho-

trexate, nourseothricin, phleomycin, triazole, and mixtures thereof. In some embodiments, the inhibitor is selected from a group consisting of bismuth (III), copper (II), and mixtures thereof.

In some embodiments, the recombinant production microorganism is engineered to express a polypeptide that increases tolerance of the host cell to the at least one compound which preferentially inhibits at least one contaminant microorganism. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to SEQ ID NO:9, or an active variant, fragment or derivative of SEQ ID NO:9. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to formaldehyde dehydrogenase (SEQ ID NO:7). In some embodiments, the polypeptide is selected from a group consisting of an amino acid sequence of at least about 80% identity to SEQ ID NO:6, an amino acid sequence of at least about 80% identity to SEQ ID NO:7, and mixtures thereof. In some embodiments, the polypeptide is selected from a group consisting of an amino acid sequence of at least about 80% identity to SEQ ID NO:11, an amino acid sequence of at least about 80% identity to SEQ ID NO:12, and mixtures thereof. In some embodiments, the polypeptide has 3-phosphoshikimate 1-carboxylvinyltransferase activity. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to 3-phosphoshikimate 1-carboxylvinyltransferase. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to SEQ ID NO:13. In some embodiments, the polypeptide is selected from a group consisting of a polypeptide that has 5-enolpyruvoylshikimate-3-phosphate synthetase (ESPS) activity and confers resistance to glyphosate, a polypeptide that has glyphosate N-acetyltransferase activity and confers resistance to glyphosate, and mixtures thereof.

In some embodiments, the polypeptide is from a bacteria of the family Enterobacteriaceae. In some embodiments, the polypeptide is from a bacterial genus selected from the group consisting of: *Alishewanella, Alterococcus, Aquamonas, Aranicola, Arsenophonus, Azotivirga, Blochmannia, Brenneria, Buchnera, Budvicia, Buttiauxella, Cedecea, Citrobacter, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Grimontella, Hafnia, Klebsiella, Kluyvera, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea, Candidatus Phlomobacter, Photorhabdus, Poodoomaamaana, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoultella, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Trabulsiella, Wigglesworthia, Xenorhabdus, Yersinia*, and *Yokenella*. In some embodiments, the polypeptide is from a microorganism of the genus *Saccharomyces*.

In some embodiments, the polypeptide is selected from a group consisting of: a polypeptide that has 5-enolpyruvoylshikimate-3-phosphate synthetase (ESPS) activity and confers resistance to glyphosate and a polypeptide that has glyphosate N-acetyltransferase activity and confers resistance to glyphosate. In some embodiments, the polypeptide is encoded by a heterologous polynucleotide.

In some embodiments, the invention is directed to a genetically modified recombinant production microorganism comprising an engineered pyruvate-utilizing pathway; and a polypeptide that increases tolerance of the host cell to inhibitors, antibiotics, or a combination thereof, wherein the production microorganism has a growth advantage over contaminant microorganisms that do not produce a desired fermentation product and do not contain said polypeptide.

In some embodiments, the recombinant production microorganism is selected from the group consisting of bacteria, cyanobacteria, filamentous fungi and yeasts. In some embodiments, the microorganism is a bacterial or cyanobacterial cell. In some embodiments, the genus of the microorganism is selected from the group consisting of *Salmonella, Arthrobacter, Bacillus, Brevibacterium, Clostridium, Corynebacterium, Gluconobacter, Nocardia, Pseudomonas, Rhodococcus, Streptomyces, Zymomonas, Escherichia, Lactobacillus, Lactococcus, Enterococcus, Alcaligenes, Klebsiella, Paenibacillus, Xanthomonas, Saccharomyces, Pichia, Hansenula, Yarrowia, Aspergillus, Kluyveromyces, Pachysolen, Rhodotorula, Zygosaccharomyces, Galactomyces, Schizosaccharomyces, Torulaspora, Debayomyces, Williopsis, Dekkera, Kloeckera, Metschnikowia*, and *Candida*.

In some embodiments, the recombinant production microorganism further comprises one or more polynucleotides that encode one or more enzymes having the following Enzyme Commission Numbers: EC 2.2.1.6, EC 1.1.1.86, EC 4.2.1.9, EC 4.1.1.72, EC 1.1.1.1, EC 1.1.1.265, EC 1.1.1.2, EC 1.2.4.4, EC 1.3.99.2, EC 1.2.1.57, EC 1.2.1.10, EC 2.6.1.66, EC 2.6.1.42, EC 1.4.1.9, EC 1.4.1.8, EC 4.1.1.14, EC 2.6.1.18, EC 2.3.1.9, EC 2.3.1.16, EC 1.1.130, EC 1.1.1.35, EC 1.1.1.157, EC 1.1.1.36, EC 4.2.1.17, EC 4.2.1.55, EC 1.3.1.44, EC 1.3.1.38, EC 5.4.99.13, EC 4.1.1.5, EC 2.7.1.29, EC 1.1.1.76, EC 1.2.1.57, and EC 4.2.1.28.

In some embodiments, the recombinant production microorganism has reduced expression of an enzyme having the following Enzyme Commission Number: EC 4.1.1.1 (pyruvate decarboxylase). In some embodiments, microorganism has reduced expression of an enzyme having the following Enzyme Commission Number: EC 1.1.1.1 (ethanol dehydrogenase).

Some embodiments are directed to a method for the production of a C3-C6 alcohol comprising the recombinant production microorganisms described herein, wherein said engineered pyruvate-utilizing pathway is a C3-C6 alcohol biosynthetic pathway; contacting said recombinant microorganism with a fermentable carbon substrate in a fermentation medium under conditions whereby a C3-C6 alcohol is produced; and recovering said C3-C6 alcohol.

In some embodiments, the C3-C6 alcohol is produced at a titer from about 5 g/L to about 100 g/L. In some embodiments, the C3-C6 alcohol is produced at a titer of at least 20 g/L. In some embodiments, the C3-C6 alcohol is selected from the group consisting of butanol, isobutanol, propanol, isopropanol, and mixtures thereof.

Some embodiments are directed to a method for the production of ethanol comprising: providing any recombinant microorganism described herein, wherein said pyruvate-utilizing pathway is an ethanol producing pathway; contacting said recombinant microorganism with a fermentable carbon substrate in a fermentation medium under conditions whereby the ethanol is produced; and recovering said ethanol.

In some embodiments, the fermentation medium comprises one or more inhibitors, antibiotics, or combinations thereof.

In some embodiments, the ethanol is produced at a titer from about 80 g/L to about 120 g/L. In some embodiments, the ethanol is produced at a titer of about 120 g/L.

Some embodiments are directed to a composition comprising any genetically modified recombinant microorganism of the invention, a fermentation medium, and one or more inhibitors, antibiotics or combinations thereof.

Some embodiments are directed to a method for reducing microbial contamination in a fermentation mix, wherein said method comprises contacting any genetically modified recombinant microorganism of the invention and a fermentation medium with one or more inhibitors, antibiotics, or mixtures thereof, and wherein the addition of more inhibitors, antibiotics, or mixtures thereof results in from about 1 log to about 10 log reduction in contamination. In some embodiments, the fermentation mix is in a propagation tank. In some embodiments, the fermentation mix is in a fermenter.

In some embodiments, reduction in contamination is measured by standard plating assays, qPCR/RT-PCR, or by measuring improved fermentation yields of desired product.

Some embodiments are directed to a method for reducing microbial contamination in a fermentation mix, wherein said method comprises contacting any genetically modified recombinant microorganism of the invention and a fermentation medium with one or more inhibitors, antibiotics, or combinations thereof, and wherein the addition of inhibitors, antibiotics, or combinations thereof results in from about 1 log to about 10 log reduction in contamination.

In some embodiments, the addition of inhibitors, antibiotics, or combinations thereof results in the death of between about 10% and about 100% of the microbial contaminants in the fermentation mix.

Some embodiments of the invention are directed to a method for reducing microbial contamination in a fermentation mix, wherein said method comprises contacting any genetically modified recombinant microorganism of the invention and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, and wherein the reduction in contamination is associated with a decrease in ethanol production. Some embodiments are directed to any composition of the invention, wherein the ethanol titer is less than about 5 g/L, or less than about 1 g/L.

Some embodiments of the invention are directed to a method for reducing microbial contamination in a fermentation mix, wherein said method comprises contacting any genetically modified recombinant microorganism of the invention and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, and wherein the reduction in contamination is associated with an increase in ethanol production.

Some embodiments are directed to a method for reducing microbial contamination in a fermentation mix, wherein said method comprises contacting any genetically modified recombinant microorganism of the invention and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, and wherein the addition of said one or more inhibitors, antibiotics, or combinations thereof results in less than an about 20% loss in the yield of a lower alkyl alcohol produced by said host cell due to the presence of microbial contaminants.

In some embodiments, the addition of said one or more inhibitors, antibiotics, or combinations thereof results in less than an about 10% loss in the yield of a lower alkyl alcohol produced by said host cell due to the presence of microbial contaminants. In some embodiments, the C3-C6 alcohol or ethanol produced is a gasoline fuel component.

Some embodiments are directed to a gasoline blend comprising about 5 to about 20% of the C3-C6 alcohol produced by the recombinant microorganisms described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts different isobutanol biosynthetic pathways. The steps labeled "a", "b", "c", "d", "e", "f", "g", "h", "i", "j", and "k" represent substrate to product conversions described below. "a" may be catalyzed, for example, by acetolactate synthase. "b" may be catalyzed, for example, by ketol-acid reductoisomerase. "c" may be catalyzed, for example, by acetohydroxy acid dehydratase. "d" may be catalyzed, for example, by branched-chain keto acid decarboxylase. "e" may be catalyzed, for example, by branched chain alcohol dehydrogenase. "f" may be catalyzed, for example, by branched chain keto acid dehydrogenase. "g" may be catalyzed, for example, by acetylating aldehyde dehydrogenase. "h" may be catalyzed, for example, by transaminase or valine dehydrogenase. "i" may be catalyzed, for example, by valine decarboxylase. "j" may be catalyzed, for example, by omega transaminase. "k" may be catalyzed, for example by isobutyryl-CoA mutase.

FIG. **2** depicts a growth inhibition assay measuring the ability of *S. cerevisiae* PNY0860-1A, PNY 827, and CEN.PK113-7D to grow in the presence of AHAS inhibitors.

FIG. **3** depicts the production of isobutanol and isobutyric acid as a function of time for the strain NYLA84.

FIG. **4** depicts the μmax of PNY 827 in dependence on concentration of copper(2+) in the medium.

FIG. **5** depicts the μmax of PNY 827 in dependence on concentration of sulfometuron methyl in the medium.

FIG. **6** depicts the μmax of PNY 827 in dependence on concentration of sulfite in the medium.

FIG. **7** depicts the μmax of PNY 827 in dependence on concentration of formaldehyde in the medium.

FIG. **8** depicts μmax of PNY 827 in dependence on concentration of 4-pyrazolecarboxylic acid in the medium.

FIG. **9** depicts μmax of PNY 827 in dependence on concentration of 4-methylpyrazole hydrochloride in the medium.

FIG. **10** depicts μmax of PNY 827 in dependence on concentration of glyoxylic acid in the medium.

FIG. **11** depicts μmax of PNY 827 in dependence on concentration of pyrazole in the medium.

FIG. **12** depicts μmax of PNY 827 in dependence on concentration of cinnamaldehyde in the medium.

FIG. **13** depicts μmax of PNY 827 in dependence on concentration of 1-bromo-2-butanone in the medium.

FIG. **14** depicts the ratio of the produced molar butanol to ethanol concentration at EPT=8 h in mixed cultures inoculated in an OD600 ratio of 1 ethanologen strain PNY 827 to 11 butanologen strain PNY 2129 in cultures without addition of an inhibitor ("Ctrl") and trans-cinnamaldehyde concentrations of 250 μM (A) and 25 mM (B), 1-bromo-2-butanone concentrations of 2 μM (A) and 200 μM (B), and pyrazole concentrations of 3 mM (A) and 30 mM (B).

FIG. **15** depicts the ratio of the produced molar butanol to ethanol concentration at EPT=8 h in mixed cultures inoculated in an OD600 ratio of 1 ethanologen strain PNY 827 to 1 butanologen strain PNY 2129 in cultures without addition of an inhibitor ("Ctrl") and trans-cinnamaldehyde at concentrations of 250 μM (A) and 25 mM (B), 1-bromo-2-butanone at concentrations of 2 μM (A) and 200 μM (B), and pyrazole at concentrations of 3 mM (A) and 30 mM (B).

FIG. **16** depicts the ratio of the produced molar butanol to ethanol concentration at EPT=48 h in mixed cultures inoculated in an OD600 ratio of 1 ethanologen strain PNY 827 to 11 butanologen strain PNY 2129 in cultures without addition of an inhibitor ("Ctrl") and trans-cinnamaldehyde at concentrations of 250 μM (A) and 25 mM (B), 1-bromo-2-butanone at concentrations of 2 μM (A) and 200 μM (B), and pyrazole at concentrations of 3 mM (A) and 30 mM (B).

FIG. **17** depicts the ratio of the produced molar butanol to ethanol concentration at EPT=48 h in mixed cultures inoculated in an OD600 ratio of 1 ethanologen strain PNY 827 to 1 butanologen strain PNY 2129 in cultures without addition of an inhibitor ("Ctrl") and trans-cinnamaldehyde at concentrations of 250 μM (A) and 25 mM (B), 1-bromo-2-butanone at concentrations of 2 μM (A) and 200 μM (B), and pyrazole at concentrations of 3 mM (A) and 30 mM (B).

FIG. **18** depicts simulated growth curves of strains A and B growing in a mixed culture at a maximum specific growth rate of 0.16 l/h and 0.61 l/h, respectively. The ratio of the biomass of strains A vs. strain B is continuously decreasing during the cultivation and is below 3% at the end of the run.

FIG. **19** depicts the predicted effect of an inhibitor c (compound) on the maximum specific growth rate of a hypothetical strain with a mumax without inhibitor addition (μ°_{max}) of 1.00 l/h, a K_r-value of 5 mM, and its behavior according a squared inhibition kinetics as described by equation (2).

DETAILED DESCRIPTION

Competition for carbon substrates in a butanologen fermentation process between the butanologen and contaminant microorganisms, such as, for example ethanol-producing yeast strains. A competitive advantage and/or selective pressure in favor of the butanologen could thus favor high yields of butanol. Such an advantage for a butanologen system may be extended to any organisms competing for the carbon substrate. The same competitive advantage may be desirable for any other recombinant production microorganism, particularly yeast competing with wildtype, ethanologen yeast and/or other microbial communities.

This invention is directed to methods employing engineered microorganisms that produce fermentation products for industrial uses, and to optimizations for producing such fermentation products at high rates and titers with advantaged economic process conditions.

Contamination by ethanologen yeast and other microbes can be problematic and can quickly lead to takeover of the fermentation, particularly when the butanologen has a slower growth rate or is otherwise less fit than the ethanologen yeast or microbe.

Applicants have solved the problem of microbial contamination by ethanologen yeast and other microbes through the use of inhibitors, antibiotics, and mixtures thereof. Butanologen yeasts either have resistance to the inhibitors, antibiotics and mixtures thereof employed, or are engineered to have resistance to the inhibitors, antibiotics, and mixtures thereof employed. The yield of the butanol process when contacted with a carbon substrate may be increased without a buildup of microbial contamination.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Also, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes.

In order to further define this invention, the following terms and definitions are herein provided.

As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers may be added to the specified method, structure, or composition.

As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. §2111.03.

Also, the indefinite articles "a" and "an" preceding an element or component of the invention are intended to be nonrestrictive regarding the number of instances, i.e., occurrences of the element or component. Therefore "a" or "an" should be read to include one or at least one, and the singular word form of the element or component also includes the plural unless the number is obviously meant to be singular.

The term "invention" or "present invention" as used herein is a non-limiting term and is not intended to refer to any single embodiment of the particular invention but encompasses all possible embodiments as described in the application.

As used herein, the term "about" modifying the quantity of an ingredient or reactant of the invention employed refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the compositions or to carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a composition resulting from a particular initial mixture. Whether or not modified by the term "about", the claims include equivalents to the quantities. In embodiments, the term "about" means within 10% of the reported numerical value, preferably within 5% of the reported numerical value.

In some instances, "biomass" as used herein refers to the cell biomass of the fermentation product-producing microorganism, typically provided in units g/L dry cell weight (dcw).

The term "fermentation product" includes any desired product of interest, including, but not limited to lactic acid, 3-hydroxy-propionic acid, acrylic acid, acetic acid, succinic acid, citric acid, fumaric acid, malic acid, itaconic acid, 1,3-propane-diol, ethylene, glycerol, isobutyrate, butanol and other lower alkyl alcohols etc.

The term "fermentation process" refers to any process by which a desired fermentation product is produced.

The term "specific growth rate", often also referred to as "μ" or "mu", is defined as $\mu=1/cx*dcx/dt$, representing the change of the biomass concentration cx in an infinitesimal short time interval dt, divided by the biomass concentration at this time.

The term "maximum specific growth rate", often also referred to as "μ_{max}" or "mumax", refers to the "specific growth rate" ("mu") during the exponential growth phase of a culture. Usually during the exponential growth phase, mu is approximately constant as the substrates are not limiting as well as the produced by-products are still not exerting a significant inhibition on growth.

The term "lower alkyl alcohol" refers to any straight-chain or branched, saturated or unsaturated, alcohol molecule with 3-6 carbon atoms.

The term "butanol" refers to 1-butanol, 2-butanol, 2-butanone, isobutanol, or mixtures thereof. Isobutanol is also known as 2-methyl-1-propanol.

The term "C3-C6 alcohol" refers to any alcohol with 3, 4, 5 or 6 carbons.

The term "butanol biosynthetic pathway" as used herein refers to an enzyme pathway to produce 1-butanol, 2-butanol, 2-butanone or isobutanol. For example, isobutanol biosynthetic pathways are disclosed in U.S. Pat. No. 7,851,188, which is incorporated by reference herein.

The term "isobutanol biosynthetic pathway" refers to the enzymatic pathway to produce isobutanol. From time to time "isobutanol biosynthetic pathway" is used synonymously with "isobutanol production pathway" (see U.S. Pat. Nos. 7,851,188 and 7,993,889, which are herein incorporated herein by reference).

The term "1-butanol biosynthetic pathway" refers to an enzymatic pathway to produce 1-butanol. A "1-butanol biosynthetic pathway" can refer to an enzyme pathway to produce 1-butanol from acetyl-coenzyme A (acetyl-CoA). For example, 1-butanol biosynthetic pathways are disclosed in U.S. Patent Application Publication No. 2008/0182308 and International Publication No. WO 2007/041269, which are herein incorporated by reference in their entireties.

The term "2-butanol biosynthetic pathway" refers to an enzymatic pathway to produce 2-butanol. A "2-butanol biosynthetic pathway" can refer to an enzyme pathway to produce 2-butanol from pyruvate. For example, 2-butanol biosynthetic pathways are disclosed in U.S. Pat. No. 8,206,970, U.S. Patent Application Publication No. 2007/0292927, International Publication Nos. WO 2007/130518 and WO 2007/130521, which are herein incorporated by reference in their entireties.

The term "2-butanone biosynthetic pathway" as used herein refers to an enzymatic pathway to produce 2-butanone (see U.S. Appl. Pub. No. 2007/0259410 and U.S. Appl. Pub. No. 2009/0155870, which are incorporated herein by reference).

The term "engineered" as used herein refers to an enzymatic pathway that is not present endogenously in a microorganism and is deliberately constructed to produce a fermentation product from a starting substrate through a series of specific substrate to product conversions.

A "recombinant microbial host cell" or a "recombinant microorganism" is defined as a host cell that has been genetically manipulated to express a biosynthetic production pathway, wherein the host cell either produces a biosynthetic product in greater quantities relative to an unmodified host cell or produces a biosynthetic product that is not ordinarily produced by an unmodified host cell. A "production microorganism" is any microorganism that produces a desired fermentation product. A "contaminant microorganism" is any microorganism that either does not produce a desired fermentation product or does produce a desired fermentation product, but at lower efficiency (for example, with lower specific productivity, rate, titer or yield) than a production microorganism. It will be appreciated that microorganisms may produce measureable amounts of more than one product, however, for the purposes herein, "product" typically refers to the major product produced by a microorganism.

The term "fermentable carbon substrate" refers to a carbon source capable of being metabolized by the microorganisms such as those disclosed herein. Suitable fermentable carbon substrates include, but are not limited to, monosaccharides, such as glucose or fructose; disaccharides, such as lactose or sucrose; oligosaccharides; polysaccharides, such as starch, cellulose, or lignocellulose, hemicellulose; one-carbon substrates, fatty acids; and any combination of these.

"Fermentation medium" as used herein means the mixture of water, sugars (fermentable carbon substrates), dissolved solids, fermentation product and all other constituents of the material in which the fermentation product is being made by the reaction of fermentable carbon substrates to fermentation products, water and carbon dioxide (CO_2) by the microorganisms present. From time to time, as used herein the term "fermentation broth", "fermentation mix" and "fermentation mixture" can be used synonymously with "fermentation medium."

The term "aerobic conditions" as used herein means growth conditions in the presence of oxygen.

The term "microaerobic conditions" as used herein means growth conditions with low levels of dissolved oxygen. For example, the oxygen level may be less than about 1% of air-saturation.

The term "anaerobic conditions" as used herein means growth conditions in the absence of oxygen.

The term "carbon substrate" refers to a carbon source capable of being metabolized by the recombinant host cells disclosed herein. Non-limiting examples of carbon substrates are provided herein and include, but are not limited to, monosaccharides, oligosaccharides, polysaccharides, ethanol, lactate, succinate, glycerol, carbon dioxide, methanol, glucose, fructose, sucrose, xylose, arabinose, dextrose, amino acids, and mixtures thereof.

The term "sucrose utilizing butanologen" as used herein refers to a microorganism capable of producing butanol from sucrose. Such microorganisms are typically recombinant microorganisms comprising an engineered butanol biosynthetic pathway. "Sucrose utilizing isobutanologen" as used herein refers to a microorganism capable of producing isobutanol from sucrose. Such microorganisms are typically recombinant microorganisms comprising an engineered isobutanol biosynthetic pathway.

As used herein, the term "yield" refers to the amount of product per amount of carbon source in g/g. The yield may be exemplified for glucose as the carbon source. It is understood unless otherwise noted that yield is expressed as a percentage of the theoretical yield. In reference to a microorganism or metabolic pathway, "theoretical yield" is defined as the maximum amount of product that can be generated per total amount of substrate as dictated by the stoichiometry of the metabolic pathway used to make the product. For example, the theoretical yield for one typical conversion of glucose to isopropanol is 0.33 g/g. As such, a yield of isopropanol from glucose of 29.7 g/g would be expressed as 90% of theoretical or 90% theoretical yield. It is understood that while in the present disclosure the yield is exemplified for glucose as a carbon source, the invention can

be applied to other carbon sources and the yield may vary depending on the carbon source used. One skilled in the art can calculate yields on various carbon sources.

The term "effective titer" as used herein, refers to the total amount of C3-C6 alcohol produced by fermentation per liter of fermentation medium. The total amount of C3-C6 alcohol includes: (i) the amount of C3-C6 alcohol in the fermentation medium; (ii) the amount of C3-C6 alcohol recovered from the organic extractant; and (iii) the amount of C3-C6 alcohol recovered from the gas phase, if gas stripping is used.

The term "effective rate" as used herein, refers to the total amount of C3-C6 alcohol produced by fermentation per liter of fermentation medium per hour of fermentation.

The term "specific productivity" as used herein, refers to the g of C3-C6 alcohol produced per g of dry cell weight of cells per unit time.

As used herein the term "coding sequence" refers to a DNA sequence that encodes for a specific amino acid sequence. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure.

The terms "derivative" and "analog" refer to a polypeptide differing from the enzymes of the invention, but retaining essential properties thereof. The term "derivative" may also refer to a host cells differing from the host cells of the invention, but retaining essential properties thereof. Generally, derivatives and analogs are overall closely similar, and, in many regions, identical to the enzymes of the invention. The terms "derived-from", "derivative" and "analog" when referring to enzymes of the invention include any polypeptides which retain at least some of the activity of the corresponding native polypeptide or the activity of its catalytic domain.

Derivatives of enzymes disclosed herein are polypeptides which may have been altered so as to exhibit features not found on the native polypeptide. Derivatives can be covalently modified by substitution (e.g. amino acid substitution), chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (e.g., a detectable moiety such as an enzyme or radioisotope). Examples of derivatives include fusion proteins, or proteins which are based on a naturally occurring protein sequence, but which have been altered. For example, proteins can be designed by knowledge of a particular amino acid sequence, and/or a particular secondary, tertiary, and/or quaternary structure. Derivatives include proteins that are modified based on the knowledge of a previous sequence, natural or synthetic, which is then optionally modified, often, but not necessarily to confer some improved function. These sequences, or proteins, are then said to be derived from a particular protein or amino acid sequence. In some embodiments of the invention, a derivative must retain at least 50% identity, at least 60% identity, at least 70% identity, at least 80% identity, at least 90% identity, at least 95% identity, at least 97% identity, or at least 99% identity to the sequence the derivative is "derived-from." In some embodiments of the invention, an enzyme is said to be derived-from an enzyme naturally found in a particular species if, using molecular genetic techniques, the DNA sequence for part or all of the enzyme is amplified and placed into a new host cell.

Polypeptides and Polynucleotides for Use in the Invention

As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis. The polypeptides used in this invention comprise full-length polypeptides and fragments thereof.

By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purposes of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

A polypeptide of the invention may be of a size of about 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

Also included as polypeptides of the present invention are derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "active variant," "active fragment," "active derivative," and "analog" refer to polypeptides of the present invention and include any polypeptides that are capable of catalyzing the reduction of a lower alkyl aldehyde. Variants of polypeptides of the present invention include polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, and/or insertions. Variants may occur naturally or be non-naturally occurring. Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions and/or additions. Derivatives of polypeptides of the present invention are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides may also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline may

be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

A "fragment" is a unique portion of a polypeptide or other enzyme used in the invention which is identical in sequence to but shorter in length than the parent full-length sequence. A fragment may comprise up to the entire length of the defined sequence, minus one amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous amino acid residues. A fragment may be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 100 or 200 amino acids of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

Alternatively, recombinant variants encoding these same or similar polypeptides can be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a host cell system.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, i.e., conservative amino acid replacements, or they can be result of replacing one amino acid with an amino acid having different structural and/or chemical properties, i.e., non-conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Alternatively, "non-conservative" amino acid substitutions can be made by selecting the differences in polarity, charge, solubility, hydrophobicity, hydrophilicity, or the amphipathic nature of any of these amino acids. "Insertions" or "deletions" are preferably in the range of about 1 to about 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

By a polypeptide having an amino acid or polypeptide sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the references sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a reference polypeptide can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., *Comp. Appi. Biosci.* 6:237-245 (1990). In a sequence alignment, the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of the global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty-0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and

C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

Polypeptides and other enzymes suitable for use in the present invention and fragments thereof are encoded by polynucleotides. The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, e.g., DNA or RNA fragments, present in a polynucleotide. Polynucleotides according to the present invention further include such molecules produced synthetically. Polynucleotides of the invention may be native to the host cell or heterologous. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid, which encodes a polypeptide normally may include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, e.g., a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide. Suitable promoters and other transcription control regions are disclosed herein.

A polynucleotide sequence can be referred to as "isolated," in which it has been removed from its native environment. For example, a heterologous polynucleotide encoding a polypeptide or polypeptide fragment having enzymatic activity (e.g., the ability to convert a substrate to xylulose) contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. An isolated polynucleotide fragment in the form of a polymer of DNA can be comprised of one or more segments of cDNA, genomic DNA, or synthetic DNA.

The term "gene" refers to a nucleic acid fragment that is capable of being expressed as a specific protein, optionally including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

As used herein, a "coding region" or "ORF" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' non-translated regions, and the like, are not part of a coding region. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence that influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences can include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures.

A variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES). In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention.

As used herein, the term "transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "recombinant" or "transformed" organisms.

The term "expression," as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

The terms "plasmid," "vector," and "cassette" refer to an extra chromosomal element often carrying genes which are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell. "Transformation cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that facilitates transformation of a particular host cell. "Expression cassette" refers to a specific vector containing a foreign gene and having elements in addition to the foreign gene that allow for enhanced expression of that gene in a foreign host.

The term "artificial" refers to a synthetic, or non-host cell derived composition, e.g., a chemically-synthesized oligonucleotide.

As used herein, "native" refers to the form of a polynucleotide, gene, or polypeptide as found in nature with its own regulatory sequences, if present.

The term "endogenous," when used in reference to a polynucleotide, a gene, or a polypeptide refers to a native polynucleotide or gene in its natural location in the genome of an organism, or for a native polypeptide, is transcribed and translated from this location in the genome.

The term "heterologous" when used in reference to a polynucleotide, a gene, or a polypeptide refers to a polynucleotide, gene, or polypeptide not normally found in the host organism. "Heterologous" also includes a native coding region, or portion thereof, that is reintroduced into the source organism in a form that is different from the corresponding native gene, e.g., not in its natural location in the organism's genome. The heterologous polynucleotide or gene may be introduced into the host organism by, e.g., gene transfer. A heterologous gene may include a native coding region with non-native regulatory regions that is reintroduced into the native host. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

The term "recombinant genetic expression element" refers to a nucleic acid fragment that expresses one or more specific proteins, including regulatory sequences preceding (5' non-coding sequences) and following (3' termination sequences) coding sequences for the proteins. A chimeric gene is a recombinant genetic expression element. The coding regions of an operon may form a recombinant genetic expression element, along with an operably linked promoter and termination region.

"Regulatory sequences" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, enhancers, operators, repressors, transcription termination signals, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure.

The term "promoter" refers to a nucleic acid sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleic acid segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". "Inducible promoters," on the other hand, cause a gene to be expressed when the promoter is induced or turned on by a promoter-specific signal or molecule. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity. For example, it will be understood that "FBA1 promoter" can be used to refer to a fragment derived from the promoter region of the FBA1 gene.

The term "terminator" as used herein refers to DNA sequences located downstream of a coding sequence. This includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of

polyadenylic acid tracts to the 3' end of the mRNA precursor. The 3' region can influence the transcription, RNA processing or stability, or translation of the associated coding sequence. It is recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical terminator activity. For example, it will be understood that "CYC1 terminator" can be used to refer to a fragment derived from the terminator region of the CYC1 gene.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of effecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "codon-optimized" as it refers to genes or coding regions of nucleic acid molecules for transformation of various hosts, refers to the alteration of codons in the gene or coding regions of the nucleic acid molecules to reflect the typical codon usage of the host organism without altering the polypeptide encoded by the DNA. Such optimization includes replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that organism.

Deviations in the nucleotide sequence that comprise the codons encoding the amino acids of any polypeptide chain allow for variations in the sequence coding for the gene. Since each codon consists of three nucleotides, and the nucleotides comprising DNA are restricted to four specific bases, there are 64 possible combinations of nucleotides, 61 of which encode amino acids (the remaining three codons encode signals ending translation). The "genetic code" which shows which codons encode which amino acids is reproduced herein as Table 1. As a result, many amino acids are designated by more than one codon. For example, the amino acids alanine and proline are coded for by four triplets, serine and arginine by six, whereas tryptophan and methionine are coded by just one triplet. This degeneracy allows for DNA base composition to vary over a wide range without altering the amino acid sequence of the proteins encoded by the DNA.

TABLE 1

The Standard Genetic Code

	T	C	A	G
T	TTT Phe (F)	TCT Ser (S)	TAT Tyr (Y)	TGT Cys (C)
	TTC Phe (F)	TCC Ser (S)	TAC Tyr (Y)	TGC
	TTA Leu (L)	TCA Ser (S)	TAA Ter	TGA Ter
	TTG Leu (L)	TCG Ser (S)	TAG Ter	TGG Trp (W)
C	CTT Leu (L)	CCT Pro (P)	CAT His (H)	CGT Arg (R)
	CTC Leu (L)	CCC Pro (P)	CAC His (H)	CGC Arg (R)
	CTA Leu (L)	CCA Pro (P)	CAA Gln (Q)	CGA Arg (R)
	CTG Leu (L)	CCG Pro (P)	CAG Gln (Q)	CGG Arg (R)
A	ATT Ile (I)	ACT Thr (T)	AAT Asn (N)	AGT Ser (S)
	ATC Ile (I)	ACC Thr (T)	AAC Asn (N)	AGC Ser (S)
	ATA Ile (I)	ACA Thr (T)	AAA Lys (K)	AGA Arg (R)
	ATG Met (M)	ACG Thr (T)	AAG Lys (K)	AGG Arg (R)
G	GTT Val (V)	GCT Ala (A)	GAT Asp (D)	GGT Gly (G)
	GTC Val (V)	GCC Ala (A)	GAC Asp (D)	GGC Gly (G)
	GTA Val (V)	GCA Ala (A)	GAA Glu (E)	GGA Gly (G)
	GTG Val (V)	GCG Ala (A)	GAG Glu (E)	GGG Gly (G)

Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Codon preference or codon bias, differences in codon usage between organisms, is afforded

by degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, inter alia, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

Given the large number of gene sequences available for a wide variety of animal, plant and microbial species, it is possible to calculate the relative frequencies of codon usage. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at the Kazusa DNA Research Institute, Japan, and these tables can be adapted in a number of ways. See Nakamura, Y., et al. *Nucl. Acids Res.* 28:292(2000). Codon usage tables for yeast, calculated from GenBank Release 128.0 [15 Feb. 2002], are reproduced below as Table 2. This table uses mRNA nomenclature, and so instead of thymine (T) which is found in DNA, the tables use uracil (U) which is found in RNA. The Table has been adapted so that frequencies are calculated for each amino acid, rather than for all 64 codons.

TABLE 2

Codon Usage Table for *Saccharomyces cerevisiae* Genes

Amino Acid	Codon	Number	Frequency per thousand
Phe	UUU	170666	26.1
Phe	UUC	120510	18.4
Leu	UUA	170884	26.2
Leu	UUG	177573	27.2
Leu	CUU	80076	12.3
Leu	CUC	35545	5.4
Leu	CUA	87619	13.4
Leu	CUG	68494	10.5
Ile	AUU	196893	30.1
Ile	AUC	112176	17.2
Ile	AUA	116254	17.8
Met	AUG	136805	20.9
Val	GUU	144243	22.1
Val	GUC	76947	11.8
Val	GUA	76927	11.8
Val	GUG	70337	10.8
Ser	UCU	153557	23.5
Ser	UCC	92923	14.2
Ser	UCA	122028	18.7
Ser	UCG	55951	8.6
Ser	AGU	92466	14.2
Ser	AGC	63726	9.8
Pro	CCU	88263	13.5
Pro	CCC	44309	6.8
Pro	CCA	119641	18.3
Pro	CCG	34597	5.3
Thr	ACU	132522	20.3
Thr	ACC	83207	12.7
Thr	ACA	116084	17.8
Thr	ACG	52045	8.0
Ala	GCU	138358	21.2
Ala	GCC	82357	12.6
Ala	GCA	105910	16.2
Ala	GCG	40358	6.2
Tyr	UAU	122728	18.8
Tyr	UAC	96596	14.8
His	CAU	89007	13.6
His	CAC	50785	7.8
Gln	CAA	178251	27.3
Gln	CAG	79121	12.1
Asn	AAU	233124	35.7
Asn	AAC	162199	24.8
Lys	AAA	273618	41.9
Lys	AAG	201361	30.8
Asp	GAU	245641	37.6
Asp	GAC	132048	20.2
Glu	GAA	297944	45.6
Glu	GAG	125717	19.2
Cys	UGU	52903	8.1
Cys	UGC	31095	4.8
Trp	UGG	67789	10.4
Arg	CGU	41791	6.4
Arg	CGC	16993	2.6
Arg	CGA	19562	3.0
Arg	CGG	11351	1.7
Arg	AGA	139081	21.3
Arg	AGG	60289	9.2
Gly	GGU	156109	23.9
Gly	GGC	63903	9.8
Gly	GGA	71216	10.9
Gly	GGG	39359	6.0
Stop	UAA	6913	1.1
Stop	UAG	3312	0.5
Stop	UGA	4447	0.7

By utilizing this or similar tables, one of ordinary skill in the art can apply the frequencies to any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide, but which uses codons optimal for a given species.

Randomly assigning codons at an optimized frequency to encode a given polypeptide sequence, can be done manually by calculating codon frequencies for each amino acid, and then assigning the codons to the polypeptide sequence randomly. Additionally, various algorithms and computer software programs are readily available to those of ordinary skill in the art. For example, the "EditSeq" function in the Lasergene Package, available from DNAstar, Inc., Madison, WI, the backtranslation function in the VectorNTI Suite, available from InforMax, Inc., Bethesda, MD, and the "backtranslate" function in the GCG--Wisconsin Package, available from Accelrys, Inc., San Diego, CA. In addition, various resources are publicly available to codon-optimize coding region sequences, e.g., the "JAVA Codon Adaptation Tool" (Grote, et al., Nucl. Acids Res. 33:W526-W531, 2005) and the "Codon optimization tool" available at Entelechon GmbH, Regensburg, Germany.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

As a practical matter, whether any particular nucleic acid molecule or polynucleotide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence or polypeptide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present

invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., *Comp. Appl. Biosci.* 6:237-245 (1990). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of the global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty-30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject nucleotide sequences, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

Standard recombinant DNA and molecular cloning techniques are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) (hereinafter "Maniatis"); and by Silhavy, T. J., Bennan, M. L. and Enquist, L. W., *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1984); and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, published by Greene Publishing Assoc. and Wiley-Interscience (1987). Additional methods used here are in *Methods in Enzymology*, Volume 194, *Guide to Yeast Genetics and Molecular and Cell Biology* (Part A, 2004, Christine Guthrie and Gerald R. Fink (Eds.), Elsevier Academic Press, San Diego, Calif.). Other molecular tools and techniques are known in the art and include splicing by overlapping extension polymerase chain reaction (PCR) (Yu, et al. (2004) Fungal Genet. Biol. 41:973-981), positive selection for mutations at the URA3 locus of *Saccharomyces cerevisiae* (Boeke, J. D. et al. (1984) Mol. Gen. Genet. 197, 345-346; M A Romanos, et al. Nucleic Acids Res. 1991 Jan. 11; 19(1): 187), the cre-lox site-specific recombination system as well as mutant lox sites and FLP substrate mutations (Sauer, B. (1987) Mol Cell Biol 7: 2087-2096; Senecoff, et al. (1988) Journal of Molecular Biology, Volume 201, Issue 2, Pages 405-421; Albert, et al. (1995) The Plant Journal. Volume 7, Issue 4, pages 649-659), "seamless" gene deletion (Akada, et al. (2006) Yeast; 23(5):399-405), and gap repair methodology (Ma et al., *Genetics* 58:201-216; 1981).

The genetic manipulations of a recombinant host cell disclosed herein can be performed using standard genetic techniques and screening and can be made in any host cell that is suitable to genetic manipulation (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202). Construction of butanologens is described herein and in the art, for example in in PCT Pub. No. WO/2012/129555, incorporated herein by reference.

"qPCR" or "RT-PCR" is a PCT-based laboratory technique that simultaneously amplifies and quantifies a target gene.

Biosynthetic Pathways

Biosynthetic pathways for the production of isobutanol that may be used include those described in U.S. Pat. Nos. 7,851,188 and 7,993,889, which are incorporated herein by reference. Isobutanol pathways are referred to with their lettering in FIG. **1**. In one embodiment, the isobutanol biosynthetic pathway comprises the following substrate to product conversions:

a) pyruvate to acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) acetolactate to 2,3-dihydroxyisovalerate, which may be catalyzed, for example, by ketol-acid reductoisomerase;
c) 2,3-dihydroxyisovalerate to α-ketoisovalerate, which may be catalyzed, for example, by dihydroxyacid dehydratase;
d) α-ketoisovalerate to isobutyraldehyde, which may be catalyzed, for example, by a branched-chain keto acid decarboxylase; and,
e) isobutyraldehyde to isobutanol, which may be catalyzed, for example, by a branched-chain alcohol dehydrogenase.

In another embodiment, the isobutanol biosynthetic pathway comprises the following substrate to product conversions:

a) pyruvate to acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) acetolactate to 2,3-dihydroxyisovalerate, which may be catalyzed, for example, by ketol-acid reductoisomerase;
c) 2,3-dihydroxyisovalerate to α-ketoisovalerate, which may be catalyzed, for example, by dihydroxyacid dehydratase;
h) α-ketoisovalerate to valine, which may be catalyzed, for example, by transaminase or valine dehydrogenase;
i) valine to isobutylamine, which may be catalyzed, for example, by valine decarboxylase;

j) isobutylamine to isobutyraldehyde, which may be catalyzed by, for example, omega transaminase; and,
e) isobutyraldehyde to isobutanol, which may be catalyzed, for example, by a branched-chain alcohol dehydrogenase.

In another embodiment, the isobutanol biosynthetic pathway comprises the following substrate to product conversions:
a) pyruvate to acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) acetolactate to 2,3-dihydroxyisovalerate, which may be catalyzed, for example, by ketol-acid reductoisomerase;
c) 2,3-dihydroxyisovalerate to α-ketoisovalerate, which may be catalyzed, for example, by dihydroxyacid dehydratase;
f) α-ketoisovalerate to isobutyryl-CoA, which may be catalyzed, for example, by branched-chain keto acid dehydrogenase;
g) isobutyryl-CoA to isobutyraldehyde, which may be catalyzed, for example, by acetylating aldehyde dehydrogenase; and,
e) isobutyraldehyde to isobutanol, which may be catalyzed, for example, by a branched-chain alcohol dehydrogenase.

In another embodiment, the isobutanol biosynthetic pathway comprises the substrate to product conversions shown as steps k, g, and e in FIG. **1**.

Biosynthetic pathways for the production of 1-butanol that may be used include those described in U.S. Appl. Pub. No. 2008/0182308, which is incorporated herein by reference. In one embodiment, the 1-butanol biosynthetic pathway comprises the following substrate to product conversions:
a) acetyl-CoA to acetoacetyl-CoA, which may be catalyzed, for example, by acetyl-CoA acetyl transferase;
b) acetoacetyl-CoA to 3-hydroxybutyryl-CoA, which may be catalyzed, for example, by 3-hydroxybutyryl-CoA dehydrogenase;
c) 3-hydroxybutyryl-CoA to crotonyl-CoA, which may be catalyzed, for example, by crotonase;
d) crotonyl-CoA to butyryl-CoA, which may be catalyzed, for example, by butyryl-CoA dehydrogenase;
e) butyryl-CoA to butyraldehyde, which may be catalyzed, for example, by butyraldehyde dehydrogenase; and,
f) butyraldehyde to 1-butanol, which may be catalyzed, for example, by butanol dehydrogenase.

Biosynthetic pathways for the production of 2-butanol that may be used include those described in U.S. Appl. Pub. No. 2007/0259410 and U.S. Appl. Pub. No. 2009/0155870, which are incorporated herein by reference. In one embodiment, the 2-butanol biosynthetic pathway comprises the following substrate to product conversions:
a) pyruvate to alpha-acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) alpha-acetolactate to acetoin, which may be catalyzed, for example, by acetolactate decarboxylase;
c) acetoin to 3-amino-2-butanol, which may be catalyzed, for example, acetoin aminase;
d) 3-amino-2-butanol to 3-amino-2-butanol phosphate, which may be catalyzed, for example, by aminobutanol kinase;
e) 3-amino-2-butanol phosphate to 2-butanone, which may be catalyzed, for example, by aminobutanol phosphate phosphorylase; and,
f) 2-butanone to 2-butanol, which may be catalyzed, for example, by butanol dehydrogenase.

In another embodiment, the 2-butanol biosynthetic pathway comprises the following substrate to product conversions:
a) pyruvate to alpha-acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) alpha-acetolactate to acetoin, which may be catalyzed, for example, by acetolactate decarboxylase;
c) acetoin to 2,3-butanediol, which may be catalyzed, for example, by butanediol dehydrogenase;
d) 2,3-butanediol to 2-butanone, which may be catalyzed, for example, by dial dehydratase; and,
e) 2-butanone to 2-butanol, which may be catalyzed, for example, by butanol dehydrogenase.

Biosynthetic pathways for the production of 2-butanone that may be used include those described in U.S. Appl. Pub. No. 2007/0259410 and U.S. Appl. Pub. No. 2009/0155870, which are incorporated herein by reference. In one embodiment, the 2-butanone biosynthetic pathway comprises the following substrate to product conversions:
a) pyruvate to alpha-acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) alpha-acetolactate to acetoin, which may be catalyzed, for example, by acetolactate decarboxylase;
c) acetoin to 3-amino-2-butanol, which may be catalyzed, for example, acetoin aminase;
d) 3-amino-2-butanol to 3-amino-2-butanol phosphate, which may be catalyzed, for example, by aminobutanol kinase; and,
e) 3-amino-2-butanol phosphate to 2-butanone, which may be catalyzed, for example, by aminobutanol phosphate phosphorylase.

In another embodiment, the 2-butanone biosynthetic pathway comprises the following substrate to product conversions:
a) pyruvate to alpha-acetolactate, which may be catalyzed, for example, by acetolactate synthase;
b) alpha-acetolactate to acetoin which may be catalyzed, for example, by acetolactate decarboxylase;
c) acetoin to 2,3-butanediol, which may be catalyzed, for example, by butanediol dehydrogenase;
d) 2,3-butanediol to 2-butanone, which may be catalyzed, for example, by dial dehydratase.

In one embodiment, the invention produces butanol from plant derived carbon sources, avoiding the negative environmental impact associated with standard petrochemical processes for butanol production. In one embodiment, the invention provides a method for the production of butanol using recombinant industrial host cells comprising a butanol pathway.

In some embodiments, the isobutanol biosynthetic pathway comprises at least one polynucleotide, at least two polynucleotides, at least three polynucleotides, or at least four polynucleotides that is/are heterologous to the host cell. In embodiments, each substrate to product conversion of an isobutanol biosynthetic pathway in a recombinant host cell is catalyzed by a heterologous polypeptide. In embodiments, the polypeptide catalyzing the substrate to product conversions of acetolactate to 2,3-dihydroxyisovalerate and/or the polypeptide catalyzing the substrate to product conversion of isobutyraldehyde to isobutanol are capable of utilizing NADH as a cofactor.

The terms "acetohydroxyacid synthase," "acetolactate synthase" and "acetolactate synthetase" (abbreviated "ALS") are used interchangeably herein to refer to an enzyme that catalyzes the conversion of pyruvate to aceto-

lactate and CO_2. Example acetolactate synthases are known by the EC number 2.2.1.6 (Enzyme Nomenclature 1992, Academic Press, San Diego). These unmodified enzymes are available from a number of sources, including, but not limited to, *Bacillus subtilis* (GenBank Nos: CAB07802.1, Z99122 (SEQ ID NO:16), NCBI (National Center for Biotechnology Information)), CAB15618), *Klebsiella pneumoniae* (GenBank Nos: AAA25079, M73842, *Lactococcus lactis* (GenBank Nos: AAA25161, L16975), *S. cerevisiae* (SEQ ID NOs:130 and 131), *E. coli* K12 (SEQ ID NOs:132 and 133).

The term "ketol-acid reductoisomerase" ("KARI"), "acetohydroxy acid reductoisomerase" and "acetohydroxy acid isomeroreductase" will be used interchangeably and refer to enzymes capable of catalyzing the reaction of (S)-acetolactate to 2,3-dihydroxyisovalerate. Example KARI enzymes may be classified as EC number EC 1.1.1.86 (Enzyme Nomenclature 1992, Academic Press, San Diego), and are available from a vast array of microorganisms, including, but not limited to, *Escherichia coli* (GenBank Nos: NP_418222, NC_000913), *Saccharomyces cerevisiae* (GenBank Nos: NP_013459, NC_001144), *Methanococcus maripaludis* (GenBank Nos: CAF30210, BX957220), and *Bacillus subtilis* (GenBank Nos: CAB14789, Z99118). KARIs include *Anaerostipes caccae* KARI variants "K9G9," "K9D3" and "K9JB4P" (SEQ ID NOs:37, 38, and 182 respectively). Ketol-acid reductoisomerase (KARI) enzymes are described in U.S. Patent Appl. Pub. Nos. 20080261230 A1, 20090163376 A1, 20100197519 A1, PCT Appl. Pub. Nos. WO/2011/041415, and WO/2012/129555, which are incorporated herein by reference. Examples of KARIs disclosed therein are those from *Lactococcus lactis, Vibrio cholera, Pseudomonas aeruginosa* PAO1, and *Pseudomonas fluorescens* PF5 mutants. *Pseudomonas fluorescens* KARIs include SEQ ID NO:134. In some embodiments, the KARI utilizes NADH. In some embodiments, the KARI utilizes NADPH. In some embodiments, the KARI utilizes NADH or NADPH.

The term "acetohydroxy acid dehydratase" and "dihydroxyacid dehydratase" ("DHAD") refers to an enzyme that catalyzes the conversion of 2,3-dihydroxyisovalerate to α-ketoisovalerate. Example acetohydroxy acid dehydratases are known by the EC number 4.2.1.9. Such enzymes are available from a vast array of microorganisms, including, but not limited to, *E. coli* (GenBank Nos: YP_026248, NC_000913), *S. cerevisiae* (GenBank Nos: NP_012550, NC 001142), *M. maripaludis* (GenBank Nos: CAF29874, BX957219), *B. subtilis* (GenBank Nos: CAB14105, Z99115), *L. lactis* (SEQ ID NO:108), and *N. crassa*. US Appl. Pub. No. 20100081154 A1, and U.S. Pat. No. 7,851, 188, which are incorporated herein by reference, describe dihydroxyacid dehydratases (DHADs), including a DHAD from *Streptococcus mutans* (SEQ ID NO:135). Example DHADs include variants of *S. mutans* DHAD, for example "L2V4" (SEQ ID NO:183).

The term "branched-chain α-keto acid decarboxylase" or "α-ketoacid decarboxylase" or "α-ketoisovalerate decarboxylase" or "2-ketoisovalerate decarboxylase" ("KIVD") refers to an enzyme that catalyzes the conversion of α-ketoisovalerate to isobutyraldehyde and CO_2. Example branched-chain α-keto acid decarboxylases are known by the EC number 4.1.1.72 and are available from a number of sources, including, but not limited to, *Lactococcus lactis* (GenBank Nos: AAS49166 (SEQ ID NO:141), AY548760; CAG34226, AJ746364, *Salmonella typhimurium* (GenBank Nos: NP_461346, NC_003197), *Clostridium acetobutylicum* (GenBank Nos: NP_149189, NC_001988), *M. caseolyticus* (SEQ ID NOs:118, 137), and *L. grayi* (SEQ ID NO:136).

The term "branched-chain alcohol dehydrogenase" ("ADH") refers to an enzyme that catalyzes the conversion of isobutyraldehyde to isobutanol. Example branched-chain alcohol dehydrogenases are known by the EC number 1.1.1.265, but may also be classified under other alcohol dehydrogenases (specifically, EC 1.1.1.1 or 1.1.1.2). Alcohol dehydrogenases may be NADPH dependent or NADH dependent. Such enzymes are available from a number of sources, including, but not limited to, *S. cerevisiae* (GenBank Nos: NP_010656, NC_001136; NP_014051, NC_001145), *E. coli* (GenBank Nos: NP_417484, NC_000913), *C. acetobutylicum* (GenBank Nos: NP_349892, NC_003030; NP_349891, NC_003030). U.S. Pat. No. 8,188,250, which is incorporated herein by reference, describes SadB, an alcohol dehydrogenase (ADH) from *Achromobacter xylosoxidans* (SEQ ID NO:139). Alcohol dehydrogenases also include horse liver ADH (SEQ ID NO:142) and *Beijerinkia indica* ADH (SEQ ID NO:138) (as described by U.S. Appl. Publ. No. 20110269199, which is incorporated herein by reference).

The term "butanol dehydrogenase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of isobutyraldehyde to isobutanol or the conversion of 2-butanone and 2-butanol. Butanol dehydrogenases are a subset of a broad family of alcohol dehydrogenases. Butanol dehydrogenase may be NAD- or NADP-dependent. The NAD-dependent enzymes are known as EC 1.1.1.1 and are available, for example, from *Rhodococcus ruber* (GenBank Nos: CAD36475, AJ491307). The NADP dependent enzymes are known as EC 1.1.1.2 and are available, for example, from *Pyrococcus furiosus* (GenBank Nos: AAC25556, AF013169). Additionally, a butanol dehydrogenase is available from *Escherichia coli* (GenBank Nos: NP_417484, NC_000913) and a cyclohexanol dehydrogenase is available from *Acinetobacter* sp. (GenBank Nos: AAG10026, AF282240). The term "butanol dehydrogenase" also refers to an enzyme that catalyzes the conversion of butyraldehyde to 1-butanol, using either NADH or NADPH as cofactor. Butanol dehydrogenases are available from, for example, *C. acetobutylicum* (GenBank NOs: NP_149325, NC_001988; note: this enzyme possesses both aldehyde and alcohol dehydrogenase activity); NP_349891, NC_003030; and NP_349892, NC_003030), *E. coli* (GenBank NOs: NP_417-484, NC_000913), and *A. xylosoxidans* (SEQ ID NOs:47 and 48, as described in U.S. Pat. No. 8,188,250, which is incorporated herein by reference in its entirety.

The term "branched-chain keto acid dehydrogenase" refers to an enzyme that catalyzes the conversion of α-ketoisovalerate to isobutyryl-CoA (isobutyryl-coenzyme A), typically using NAD^+ (nicotinamide adenine dinucleotide) as an electron acceptor. Example branched-chain keto acid dehydrogenases are known by the EC number 1.2.4.4. Such branched-chain keto acid dehydrogenases are comprised of four subunits and sequences from all subunits are available from a vast array of microorganisms, including, but not limited to, *B. subtilis* (GenBank Nos: CAB14336, Z99116; CAB14335, Z99116; CAB14334, Z99116; and CAB14337, Z99116) and *Pseudomonas putida* (GenBank Nos: AAA65614, M57613; AAA65615, M57613; AAA65617, M57613; and AAA65618, M57613).

The term "acylating aldehyde dehydrogenase" refers to an enzyme that catalyzes the conversion of isobutyryl-CoA to isobutyraldehyde, typically using either NADH or NADPH as an electron donor. Example acylating aldehyde dehydro-

genases are known by the EC numbers 1.2.1.10 and 1.2.1.57. Such enzymes are available from multiple sources, including, but not limited to, *Clostridium beijerinckii* (GenBank Nos: AAD31841, AF157306), *C. acetobutylicum* (GenBank Nos: NP_149325, NC_001988; NP_149199, NC_001988), *P. putida* (GenBank Nos: AAA89106, U13232), and *Thermus thermophilus* (GenBank Nos: YP_145486, NC_006461).

The term "transaminase" refers to an enzyme that catalyzes the conversion of α-ketoisovalerate to L-valine, using either alanine or glutamate as an amine donor. Example transaminases are known by the EC numbers 2.6.1.42 and 2.6.1.66. Such enzymes are available from a number of sources. Examples of sources for alanine-dependent enzymes include, but are not limited to, *E. coli* (GenBank Nos: YP_026231, NC_000913) and *Bacillus licheniformis* (GenBank Nos: YP_093743, NC_006322). Examples of sources for glutamate-dependent enzymes include, but are not limited to, *E. coli* (GenBank Nos: YP_026247, NC_000913), *S. cerevisiae* (GenBank Nos: NP_012682, NC_001142) and *Methanobacterium thermoautotrophicum* (GenBank Nos: NP_276546, NC_000916).

The term "valine dehydrogenase" refers to an enzyme that catalyzes the conversion of α-ketoisovalerate to L-valine, typically using NAD(P)H as an electron donor and ammonia as an amine donor. Example valine dehydrogenases are known by the EC numbers 1.4.1.8 and 1.4.1.9 and such enzymes are available from a number of sources, including, but not limited to, *Streptomyces coelicolor* (GenBank Nos: NP_628270, NC_003888) and *B. subtilis* (GenBank Nos: CAB14339, Z99116).

The term "valine decarboxylase" refers to an enzyme that catalyzes the conversion of L-valine to isobutylamine and CO_2. Example valine decarboxylases are known by the EC number 4.1.1.14. Such enzymes are found in *Streptomyces*, such as for example, *Streptomyces viridifaciens* (GenBank Nos: AAN10242, AY116644).

The term "omega transaminase" refers to an enzyme that catalyzes the conversion of isobutylamine to isobutyraldehyde using a suitable amino acid as an amine donor. Example omega transaminases are known by the EC number 2.6.1.18 and are available from a number of sources, including, but not limited to, *Alcaligenes denitrificans* (AAP92672, AY330220), *Ralstonia eutropha* (GenBank Nos: YP_294474, NC_007347), *Shewanella oneidensis* (GenBank Nos: NP_719046, NC_004347), and *P. putida* (GenBank Nos: AAN66223, AE016776).

The term "acetyl-CoA acetyltransferase" refers to an enzyme that catalyzes the conversion of two molecules of acetyl-CoA to acetoacetyl-CoA and coenzyme A (CoA). Example acetyl-CoA acetyltransferases are acetyl-CoA acetyltransferases with substrate preferences (reaction in the forward direction) for a short chain acyl-CoA and acetyl-CoA and are classified as E.C. 2.3.1.9 [Enzyme Nomenclature 1992, Academic Press, San Diego]; although, enzymes with a broader substrate range (E.C. 2.3.1.16) will be functional as well. Acetyl-CoA acetyltransferases are available from a number of sources, for example, *Escherichia coli* (GenBank Nos: NP_416728, NC_000913; NCBI (National Center for Biotechnology Information) amino acid sequence, NCBI nucleotide sequence), *Clostridium acetobutylicum* (GenBank Nos: NP_349476.1, NC_003030; NP_149242, NC_001988, *Bacillus subtilis* (GenBank Nos: NP_390297, NC_000964), and *Saccharomyces cerevisiae* (GenBank Nos: NP_015297, NC_001148).

The term "3-hydroxybutyryl-CoA dehydrogenase" refers to an enzyme that catalyzes the conversion of acetoacetyl-CoA to 3-hydroxybutyryl-CoA. Example hydroxybutyryl-CoA dehydrogenases may be reduced nicotinamide adenine dinucleotide (NADH)-dependent, with a substrate preference for (S)-3-hydroxybutyryl-CoA or (R)-3-hydroxybutyryl-CoA. Examples may be classified as E.C. 1.1.1.35 and E.C. 1.1.1.30, respectively. Additionally, 3-hydroxybutyryl-CoA dehydrogenases may be reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent, with a substrate preference for (S)-3-hydroxybutyryl-CoA or (R)-3-hydroxybutyryl-CoA and are classified as E.C. 1.1.1.157 and E.C. 1.1.1.36, respectively. 3-Hydroxybutyryl-CoA dehydrogenases are available from a number of sources, for example, *C. acetobutylicum* (GenBank NOs: NP_349314, NC_003030), *B. subtilis* (GenBank NOs: AAB09614, U29084), *Ralstonia eutropha* (GenBank NOs: YP_294481, NC_007347), and *Alcaligenes eutrophus* (GenBank NOs: AAA21973, J04987).

The term "crotonase" refers to an enzyme that catalyzes the conversion of 3-hydroxybutyryl-CoA to crotonyl-CoA and H_2O. Example crotonases may have a substrate preference for (S)-3-hydroxybutyryl-CoA or (R)-3-hydroxybutyryl-CoA and may be classified as E.C. 4.2.1.17 and E.C. 4.2.1.55, respectively. Crotonases are available from a number of sources, for example, *E. coli* (GenBank NOs: NP_415911, NC_000913), *C. acetobutylicum* (GenBank NOs: NP_349318, NC_003030), *B. subtilis* (GenBank NOs: CAB13705, Z99113), and *Aeromonas caviae* (GenBank NOs: BAA21816, D88825).

The term "butyryl-CoA dehydrogenase" refers to an enzyme that catalyzes the conversion of crotonyl-CoA to butyryl-CoA. Example butyryl-CoA dehydrogenases may be NADH-dependent, NADPH-dependent, or flavin-dependent and may be classified as E.C. 1.3.1.44, E.C. 1.3.1.38, and E.C. 1.3.99.2, respectively. Butyryl-CoA dehydrogenases are available from a number of sources, for example, *C. acetobutylicum* (GenBank NOs: NP_347102, NC_003030), *Euglena gracilis* (GenBank NOs: Q5EU90), AY741582), *Streptomyces collinus* (GenBank NOs: AAA92890, U37135), and *Streptomyces coelicolor* (GenBank NOs: CAA22721, AL939127).

The term "butyraldehyde dehydrogenase" refers to an enzyme that catalyzes the conversion of butyryl-CoA to butyraldehyde, using NADH or NADPH as cofactor. Butyraldehyde dehydrogenases with a preference for NADH are known as E.C. 1.2.1.57 and are available from, for example, *Clostridium beijerinckii* (GenBank NOs: AAD31841, AF157306) and *C. acetobutylicum* (GenBank NOs: NP_149325, NC_001988).

The term "isobutyryl-CoA mutase" refers to an enzyme that catalyzes the conversion of butyryl-CoA to isobutyryl-CoA. This enzyme uses coenzyme B_{12} as cofactor. Example isobutyryl-CoA mutases are known by the EC number 5.4.99.13. These enzymes are found in a number of *Streptomyces*, including, but not limited to, *Streptomyces cinnamonensis* (GenBank Nos: AAC08713, U67612; CAB59633, AJ246005), *S. coelicolor* (GenBank Nos: CAB70645, AL939123; CAB92663, AL939121), and *Streptomyces avermitilis* (GenBank Nos: NP_824008, NC_003155; NP_824637, NC_003155).

The term "acetolactate decarboxylase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of alpha-acetolactate to acetoin. Example acetolactate decarboxylases are known as EC 4.1.1.5 and are available, for example, from *Bacillus subtilis* (GenBank Nos: AAA22223, L04470), *Klebsiella terrigena* (GenBank Nos: AAA25054, L04507) and *Klebsiella pneumoniae* (GenBank Nos: AAU43774, AY722056).

The term "acetoin aminase" or "acetoin transaminase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of acetoin to 3-amino-2-butanol. Acetoin aminase may utilize the cofactor pyridoxal 5'-phosphate or NADH (reduced nicotinamide adenine dinucleotide) or NADPH (reduced nicotinamide adenine dinucleotide phosphate). The resulting product may have (R) or (S) stereochemistry at the 3-position. The pyridoxal phosphate-dependent enzyme may use an amino acid such as alanine or glutamate as the amino donor. The NADH- and NADPH-dependent enzymes may use ammonia as a second substrate. A suitable example of an NADH dependent acetoin aminase, also known as amino alcohol dehydrogenase, is described by Ito et al. (U.S. Pat. No. 6,432,688). An example of a pyridoxal-dependent acetoin aminase is the amine:pyruvate aminotransferase (also called amine:pyruvate transaminase) described by Shin and Kim (*J. Org. Chem.* 67:2848-2853 (2002)).

The term "acetoin kinase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of acetoin to phosphoacetoin. Acetoin kinase may utilize ATP (adenosine triphosphate) or phosphoenolpyruvate as the phosphate donor in the reaction. Enzymes that catalyze the analogous reaction on the similar substrate dihydroxyacetone, for example, include enzymes known as EC 2.7.1.29 (Garcia-Alles et al. (2004) Biochemistry 43:13037-13046).

The term "acetoin phosphate aminase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of phosphoacetoin to 3-amino-2-butanol O-phosphate. Acetoin phosphate aminase may use the cofactor pyridoxal 5'-phosphate, NADH or NADPH. The resulting product may have (R) or (S) stereochemistry at the 3-position. The pyridoxal phosphate-dependent enzyme may use an amino acid such as alanine or glutamate. The NADH and NADPH-dependent enzymes may use ammonia as a second substrate. Although there are no reports of enzymes catalyzing this reaction on phosphoacetoin, there is a pyridoxal phosphate-dependent enzyme that is proposed to carry out the analogous reaction on the similar substrate serinol phosphate (Yasuta et al. (2001) Appl. Environ. Microbial. 67:4999-5009.

The term "aminobutanol phosphate phospholyase", also called "amino alcohol O-phosphate lyase", refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 3-amino-2-butanol O-phosphate to 2-butanone. Amino butanol phosphate phospho-lyase may utilize the cofactor pyridoxal 5'-phosphate. There are reports of enzymes that catalyze the analogous reaction on the similar substrate 1-amino-2-propanol phosphate (Jones et al. (1973) Biochem J. 134:167-182). U.S. Appl. Pub. No. 2007/0259410 describes an aminobutanol phosphate phospho-lyase from the organism *Erwinia carotovora.*

The term "aminobutanol kinase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 3-amino-2-butanol to 3-amino-2butanol O-phosphate. Amino butanol kinase may utilize ATP as the phosphate donor. Although there are no reports of enzymes catalyzing this reaction on 3-amino-2-butanol, there are reports of enzymes that catalyze the analogous reaction on the similar substrates ethanolamine and 1-amino-2-propanol (Jones et al., supra). U.S. Appl. Pub. No. 2009/0155870 describes, in Example 14, an amino alcohol kinase of *Erwinia carotovora* subsp. *Atroseptica.*

The term "butanediol dehydrogenase" also known as "acetoin reductase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of acetoin to 2,3-butanediol. Butanediol dehydrogenases are a subset of the broad family of alcohol dehydrogenases. Butanediol dehydrogenase enzymes may have specificity for production of (R)- or (S)-stereochemistry in the alcohol product. (S)-specific butanediol dehydrogenases are known as EC 1.1.1.76 and are available, for example, from *Klebsiella pneumoniae* (GenBank Nos: BBA13085, D86412). (R)-specific butanediol dehydrogenases are known as EC 1.1.1.4 and are available, for example, from *Bacillus cereus* (GenBank Nos. NP 830481, NC_004722; AAP07682, AE017000), and *Lactococcus lactis* (GenBank Nos. AAK04995, AE006323).

The term "butanediol dehydratase", also known as "dial dehydratase" or "propanediol dehydratase" refers to a polypeptide (or polypeptides) having an enzyme activity that catalyzes the conversion of 2,3-butanediol to 2-butanone. Butanediol dehydratase may utilize the cofactor adenosyl cobalamin (also known as coenzyme Bw or vitamin B12; although vitamin B12 may refer also to other forms of cobalamin that are not coenzyme B12). Adenosyl cobalamin-dependent enzymes are known as EC 4.2.1.28 and are available, for example, from *Klebsiella oxytoca* (GenBank Nos: AA08099 (alpha subunit), D45071; BAA08100 (beta subunit), D45071; and BBA08101 (gamma subunit), D45071 (Note all three subunits are required for activity), and *Klebsiella pneumonia* (GenBank Nos: AAC98384 (alpha subunit), AF102064; GenBank Nos: AAC98385 (beta subunit), AF102064, GenBank Nos: AAC98386 (gamma subunit), AF102064). Other suitable dial dehydratases include, but are not limited to, B12-dependent dial dehydratases available from *Salmonella typhimurium* (GenBank Nos: AAB84102 (large subunit), AF026270; GenBank Nos: AAB84103 (medium subunit), AF026270; GenBank Nos: AAB84104 (small subunit), AF026270); and *Lactobacillus collinoides* (GenBank Nos: CAC82541 (large subunit), AJ297723; GenBank Nos: CAC82542 (medium subunit); AJ297723; GenBank Nos: CAD01091 (small subunit), AJ297723); and enzymes from *Lactobacillus brevis* (particularly strains CNRZ 734 and CNRZ 735, Speranza et al., J. Agric. Food Chem. (1997) 45:3476-3480), and nucleotide sequences that encode the corresponding enzymes. Methods of dial dehydratase gene isolation are well known in the art (e.g., U.S. Pat. No. 5,686,276).

The term "pyruvate decarboxylase" refers to an enzyme that catalyzes the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. Pyruvate decarboxylases are known by the EC number 4.1.1.1. These enzymes are found in a number of yeast, including *Saccharomyces cerevisiae* (GenBank Nos: CAA97575 (SEQ ID NO:1), CAA97705 (SEQ ID NO:2), CAA97091 (SEQ ID NO:3)).

The term "ethanol dehydrogenase" or "alcohol dehydrogenase" refers to an enzyme that catalyze the interconversion between aldehydes or ketones and alcohols, frequently using either NADH and/or NADPH as cofactors. Ethanol dehydrogenases comprise the EC numbers 1.1.1.1., 1.1.99.8., 1.1.1.244., 1.1.2.B1., 1.1.2.B2., 1.1.2.B3.

It will be appreciated that host cells comprising an isobutanol biosynthetic pathway as provided herein may further comprise one or more additional modifications. U.S. Appl. Pub. No. 20090305363 (incorporated by reference) discloses increased conversion of pyruvate to acetolactate by engineering yeast for expression of a cytosol-localized acetolactate synthase and substantial elimination of pyruvate decarboxylase activity. In some embodiments, the host cells comprise modifications to reduce glycerol-3-phosphate dehydrogenase activity and/or disruption in at least one gene encoding a polypeptide having pyruvate decarboxylase

activity or a disruption in at least one gene encoding a regulatory element controlling pyruvate decarboxylase gene expression as described in U.S. Patent Appl. Pub. No. 20090305363 (incorporated herein by reference), modifications to a host cell that provide for increased carbon flux through an Entner-Doudoroff Pathway or reducing equivalents balance as described in U.S. Patent Appl. Pub. No. 20100120105 (incorporated herein by reference). Other modifications are described in PCT Pub. No. WO/2012/129555, incorporated herein by reference, and include integration of at least one polynucleotide encoding a polypeptide that catalyzes a step in a pyruvate-utilizing biosynthetic pathway. Other modifications include at least one deletion, mutation, and/or substitution in an endogenous polynucleotide encoding a polypeptide having acetolactate reductase activity. In embodiments, the polypeptide having acetolactate reductase activity is YMR226C (SEQ ID NOs:4, 5) of *Saccharomyces cerevisiae* or a homolog thereof. Additional modifications include a deletion, mutation, and/or substitution in an endogenous polynucleotide encoding a polypeptide having aldehyde dehydrogenase and/or aldehyde oxidase activity. In embodiments, the polypeptide having aldehyde dehydrogenase activity is ALD6 from *Saccharomyces cerevisiae* or a homolog thereof. A genetic modification which has the effect of reducing glucose repression wherein the yeast production host cell is pdc- is described in U.S. Appl. Publication No. 20110124060, incorporated herein by reference. In some embodiments, the pyruvate decarboxylase that is deleted or downregulated is selected from the group consisting of: PDC1, PDC5, PDC6, and combinations thereof. In some embodiments, the pyruvate decarboxylase is selected from those enzymes described in U.S. Patent Appl. Pub. No. 20090305363. In some embodiments, host cells contain a deletion or downregulation of a polynucleotide encoding a polypeptide that catalyzes the conversion of glyceraldehyde-3-phosphate to glycerate 1,3, bisphosphate. In some embodiments, the enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase.

Yeasts may have one or more genes encoding pyruvate decarboxylase. For example, there is one gene encoding pyruvate decarboxylase in *Candida glabrata* and *Schizosaccharomyces pombe*, while there are three isozymes of pyruvate decarboxylase encoded by the PDC1, PCD5, and PDC6 genes in *Saccharomyces*. In some embodiments, in the present yeast cells at least one PDC gene is inactivated. If the yeast cell used has more than one expressed (active) PDC gene, then each of the active PDC genes may be modified or inactivated thereby producing a pdc− cell. For example, in *S. cerevisiae* the PDC1, PDC5, and PDC6 genes may be modified or inactivated. If a PDC gene is not active under the fermentation conditions to be used then such a gene would not need to be modified or inactivated.

Other target genes, such as those encoding pyruvate decarboxylase proteins having at least 70-75%, at least 75-80%, at least 80-85%, at least 85%-90%, at least 90%-95%, or at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the pyruvate decarboxylases described in U.S. Patent Appl. Pub. No. 20090305363 may be identified in the literature and in bioinformatics databases well known to the skilled person.

Recombinant host cells may further comprise (a) at least one heterologous polynucleotide encoding a polypeptide having dihydroxy-acid dehydratase activity; and (b)(i) at least one deletion, mutation, and/or substitution in an endogenous gene encoding a polypeptide affecting Fe—S cluster biosynthesis; and/or (ii) at least one heterologous polynucleotide encoding a polypeptide affecting Fe—S cluster biosynthesis described in U.S. Patent Appl. Pub. No. US20120064561, incorporated herein by reference. In embodiments, the polypeptide affecting Fe—S cluster biosynthesis is encoded by AFT1, AFT2, FRA2, GRX3 or CCC1. AFT1 and AFT2 are described by WO/2001/103300, which is incorporated herein by reference. In embodiments, the polypeptide affecting Fe—S cluster biosynthesis is constitutive mutant AFT1 L99A, AFT1 L102A, AFT1 C291F, or AFT1 C293F.

In some embodiments, the host cell further comprises one or more polynucleotides that encode one or more enzymes having the following Enzyme Commission Numbers: EC 4.1.1.1 (PDC1, 5, and 6) (SEQ ID NOs:1, 2, and 3) and EC 1.1.1.1 (alcohol dehydrogenase).

In some embodiments of the invention, there are one or more inhibitors, antibiotics, or combinations thereof in the fermentation medium.

In some embodiments, the inhibitor is an inhibitor of an ethanol biosynthesis pathway. In some embodiments, the inhibitor inhibits pyruvate decarboxylase and/or alcohol dehydrogenase. In some embodiments, the inhibitor is selected from the group consisting of: the XC_6H_4CH=CHCOCOOH class of inhibitors/substrate analogues, cinnamaldehydes, glyoxalic acid, ketomalonate, regulatory site inhibitors, p-chloromercuribenzoic acid (pCMB), 5,5'-dithiobis(2-nitrobenzoic acid) (DNTB), pyrazole, 4-pyrazolecarboxylic acid, 1-H -pyrazole-1-carboxamidine-HC1, 4-methylpyrazole, 1-bromo-2-butanone, pyrazole-3,5-dicarboxylic acid monohydrate, and mixtures thereof. In some embodiments, the XC_6H_4CH=CHCOCOOH inhibitors/substrate analogue is CPB((E)-4-(4-chlorophenyl)-2-oxo-3-butenoic acid. In some embodiments the cinnamaldehyde is p-nitrocinnamaldehyde (NA). In some embodiments, the regulatory site inhibitors are iodoacetate, 1,3-dibromoacetone, 1-bromo-2-butanone. "Cinnamaldehyde" includes both trans-cinnamaldehydes and 4-nitrocinnamaldehydes. In some embodiments, copper (II) is added at a concentration of at least about 1.1 mM, at least about 11 mM, at least about 33 mM. In some embodiments, sulfometuron methyl is added at a concentration of at least about 0.001 mM, at least about 0.01 mM, at least about 0.1 mM. In some embodiments, sulfite is added at a concentration of at least about 0.6 mM, at least about 6.2 mM, at least about 62 mM. In some embodiments, formaldehyde is added at a concentration of at least about 0.09 mM, at least about 0.9 mM, at least about 2.7 mM. In some embodiments, pyrazole is added at a concentration of at least about 0.3 mM, at least about 3 mM, at least about 30 mM. In some embodiments, 4-methylpyrazole hydrochloride is added at a concentration of at least about 4.1 mM, at least about 41mM, at least about 123 mM. In some embodiments, 4-pyrazolecarboxylic acid is added at a concentration of at least about 10 mM, at least about 100 mM, at least about 300 mM. In some embodiments, 1-bromo-2-butanone is added at a concentration of at least about 0.0002 mM, at least about 0.002 mM, at least about 0.006 mM. In some embodiments, trans-cinnamaldehyde is added at a concentration of at least about 0.025 mM, at least about 0.25 mM, at least about 0.75 mM. In some embodiments, glyoxylic acid is added at a concentration of at least about 16.8 mM, at least about 168 mM, at least about 504 mM.

In some embodiments, the inhibitor is a chemical. In some embodiments, the chemical is selected from the group consisting of: fluoroacetate (dehH1), fluorophenyalanine, formaldehyde (SFA1), sulfite (FZF1-4), and trifluoroleucine (LEU4-1).

In some embodiments, the inhibitor is an inhibitor of an amino acid biosynthesis pathway. In some embodiments, the inhibitor is an acetohydroxy acid synthase (AHAS) inhibitor. In some embodiments, the inhibitor is a sulfonylurea herbicide. In some embodiments, the sulfonylurea herbicide is selected from the group consisting of: imidazolinones, triazolopyrimidines, pyrimidinyl oxybenzoates, sulfonylureas, sulfonylamino carbonyl triazolinones, and mixtures thereof. In some embodiments, the inhibitor is selected from the group consisting of: nicosulfuron methyl, metsulfuron methyl, chlorimuron ethyl, sulfometuron methyl, chlorsulfuron, thifensulfuron methyl, and mixtures thereof. In some embodiments, the sulfonylurea herbicide is an acetohydroxyacid synthase (AHAS) inhibitor).

In some embodiments, resistance to the sulfonyl urea is conferred by a polypeptide encoded by a heterologous polynucleotide. In some embodiments, the heterologous polynucleotide provides resistance to AHAS inhibitors and comprises a sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NO:130 (ILV2 gene from *S. cerevisiae* BY4700) and SEQ ID NO:132 (ALS I gene from *E. coli* K12). In some embodiments, the heterologous polypeptide provides resistance to AHAS inhibitors and comprises an amino acid sequence having at least 80% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:131 (ILV2 from *S. cerevisiae* BY4700) and SEQ ID NO:133 (ALS I from *E. coli* K12). In some embodiments, the polypeptide provides resistance to AHAS inhibitors and comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:131 (ILV2 from *S. cerevisiae* BY4700) and SEQ ID NO:133 (ALS I gene from *E. coli* K12) or an active variant, fragment or derivative thereof. In some embodiments, the polypeptide is from a bacteria of the family Enterobacteriaceae. In some embodiments, the polypeptide is from a bacterial genus selected from the group consisting of: *Alishewanella, Alterococcus, Aquamonas, Aranicola, Arsenophonus, Azotivirga, Blochmannia, Brenneria, Buchnera, Budvicia, Buttiauxella, Cedecea, Citrobacter, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Grimontella, Hafnia, Klebsiella, Kluyvera, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea, Candidatus Phlomobacter, Photorhabdus, Poodoomaamaana, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoultella, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Trabulsiella, Wigglesworthia, Xenorhabdus, Yersinia*, and *Yokenella*. In some embodiments, the polypeptide is from a microorganism of the genus *Saccharomyces*. In some embodiments, the AHAS enzymes can be mutated to confer sulfonyl urea resistance. In some embodiments, the *B. subtilis* AlsS enzyme is mutated to increase its sulfonyl urea resistance.

In some embodiments, the inhibitor of amino acid synthesis is glyphosate. In some embodiments, resistance to the glyphosate is conferred by a polypeptide that has 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS) activity. In some embodiments, the polypeptide is encoded by a heterologous polynucleotide. In some embodiments, the inhibitor is a 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS) inhibitor. In some embodiments, the inhibitor is a glyphosate derivative. In some embodiments, resistance to the glyphosate is conferred by a polypeptide that has glyphosate N-acetyltransferase activity. In some embodiments, the polypeptide is encoded by a heterologous polynucleotide. Sequences describing polypeptides with glyphosate N-acetyltransferase activity are described in, for example, U.S. Pat. No. 7,863,503, which is incorporated herein by reference.

In some embodiments, the antibiotic is selected from the group consisting of: aureobasidin A, bialaphos, cerulenin, chloramphenicol, cyclohexamide, geneticin/G418, hygromycin B, methotrexate, nourseothricin, phleomycin, triazole, and mixtures thereof. In some embodiments, a polypeptide confers resistance to one or more antibiotics. In some embodiments, the polypeptide is encoded by a heterologous polynucleotide.

In some embodiments a polypeptide confers resistance to the inhibitor or antibiotic. In some embodiments, the polypeptide is encoded by a polynucleotide. In some embodiments, the polypeptide conferring resistance to the inhibitor or antibiotic has one or more amino acid deletions when compared with the amino acid sequence of the corresponding native polypeptide. In some embodiments, the amino acid sequence of the polypeptide has one or more amino acid substitutions when compared with the amino acid sequence of the corresponding native polypeptide.

In some embodiments, the inhibitor is an α-isopropyl malate (a-IPM) synthase inhibitor. In some embodiments, the inhibitor is trifluoroleucine or a trifluoroleucine derivative. In some embodiments, the inhibitor is a 3-deoxy-D-arabino-heptolusonate-7-phosphate synthase (DAHPS) inhibitor. In some embodiments, the inhibitor is fluorophenyalanine or a fluorophenyalanine derivative. In some embodiments, the inhibitor is bismuth (III) or copper (II).

In some embodiments, the polypeptide confers tolerance to fluoroacetate. In some embodiments, the polypeptide confers tolerance to formaldehyde. In some embodiments, the polypeptide confers tolerance to sulfite.

In some embodiments, the polypeptide confers tolerance to an α-isopropyl malate (a-IPM) synthase inhibitor. In some embodiments, the polypeptide confers tolerance to trifluoroleucine or a trifluoroleucine derivative (isopropyl malate resistance). In some embodiments, the polynucleotide sequence encoding the polypeptide providing resistance to trifluoroleucine comprises a sequence having at least 80% identity to a sequence disclosed by: Chianelli, M. S., et al., *Cell. Mol. Biol.* 42(6):847-57 (1996) or Oba, T., et al., *Biosci. Biotechnol. Biochem.* 70(7):1776-9 (2006) and incorporated by reference. In some embodiments, the polypeptide confers tolerance to a 3-deoxy-D-arabino-heptolusonate-7-phosphate synthase (DAHPS) inhibitor. In some embodiments, the polynucleotide sequence encoding the polypeptide providing resistance to DAHPS comprises a sequence having at least 80% identity to a sequence disclosed by: Fukada, K., et al., *Agric. Biol. Chem.* 54:3151-3156 (1990); Meuris, P. 1974. *Genetics* 76:735-744 (1974); Shimura, K., et al., 1993. *Enzyme Microbiol. Technol.* 15:874-876 (1993) and incorporated by reference.

In some embodiments, the polypeptide confers tolerance to an antibiotic. In some embodiments, the polypeptide confers tolerance to an antibiotic selected from the group consisting of: aureobasidin A, bialaphos, cerulenin, chloramphenicol, cyclohexamide, geneticin, hygromycin B, methotrexate, nourseothricin, phleomycin, triazole, and mixtures thereof. In some embodiments, the polynucleotide sequence encoding the polypeptide comprises a sequence having at least 80% identity to a sequence selected from the group consisting of: SEQ ID NOs: 92 and 143-157 (Aureobasidin A resistance (AUR1-C) (SEQ ID NOs:143 and 144); bialiphos resistance protein (SEQ ID NOs:145 and 146); cerulenin resistance YML007W Chr 13 (SEQ ID NOs:147 and 148); Geneticin resistance (kanMX) (SEQ ID

NOs:149 and 150); Hygromycin B resistance (HygR) (SEQ ID NOs:151 and 152); *Streptomyces noursei* nourseothricin resistance (natl) (SEQ ID NOs:153 and 154); phleomycin/zeocin binding protein (SEQ ID NOs:155 and 156); and Triazole resistance (cyp51A) (SEQ ID NOs:157 and 92).

In some embodiments, the inhibitor is inhibiting at least one enzyme selected from the group consisting of: 5-enolpyruvoyl-shikimate-3-phosphate synthetase, α-isopropyl malate synthase, 3-deoxy-D-arabino-heptolusonate-7-phosphate synthase and mixtures thereof. In some embodiments, the polynucleotide sequence encoding the polypeptide providing resistance to 5-enolpyruvoyl -shikimate-3-phosphate synthetase comprises a sequence having at least 80% identity to a sequence disclosed by: Cao G, et al., (2012) *PLoS ONE* 7(6): e38718 (2012) incorporated by reference. In some embodiments, the polynucleotide sequence encoding the polypeptide with glyphosate N-acetyltransferase activity comprises a sequence having at least 80% identity to a sequence disclosed by U.S. Pat. No. 7,666,644, which is incorporated herein by reference in its entirety and Siehl D.L., et al., *Pest Manag Sci.* 61(3):235-40 (2005) incorporated by reference. In some embodiments, the polynucleotide sequence encoding the polypeptide providing resistance to 3-phosphoshikimate 1-carboxylvinyltransferase comprises a sequence having at least 80% identity to a sequence disclosed by: Vande Berg B.J., et al., *Pest Manag Sci.* 64(4):340-5 (2008) incorporated by reference. In some embodiments, the polypeptide that provides resistance to the inhibitor is a formaldehyde dehydrogenase. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to SEQ ID NO:6 or 7.

In some embodiments, the polypeptide that confers resistance comprises an amino acid sequence of at least about 80% identity to SEQ ID NO:11 or SEQ ID NO:12. In some embodiments, the polypeptide that confers resistance is a 3-phosphoshikimate 1-carboxylvinyltransferase. In some embodiments, the polypeptide comprises an amino acid sequence of at least about 80% identity to SEQ ID NO:13.

In some embodiments, one or more AHAS inhibitors is present at a concentration from about 0.1 g/mL to about 2 g/mL, about 1.0 g/mL to about 0.1 g/mL, about 1 mg/mL to about 0.1 g/mL, or about 10 mg/mL to about 100 mg/mL. In some embodiments, one or more AHAS inhibitors is present at a concentration of 0.0125 mg/mL. In some embodiments, one or more AHAS inhibitors is present at a concentration of 1 mg/mL. In some embodiments, one or more AHAS inhibitors is present at a concentration of 2 mg/mL.

In some embodiments, glyphosate is at a concentration from about 0.1 μg/mL to about 2 g/mL, for example about 10 μg/mL, about 100 μg/mL, about 1 mg/mL, about 10 mg/mL, about 100 mg/mL, about 1 g/mL, or about 2 g/mL.

In some embodiments, the antibiotic is present at a concentration from about 2 ppm to about 500 ppm, for example about 5 ppm, about 20 ppm, about 50 ppm, about 100 ppm, about 150 ppm, about 200 ppm, about 300 ppm, about 400 ppm, or about 500 ppm.

In some embodiments, the addition inhibitors of amino acid synthesis, antibiotics, or combinations thereof results in death of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the microbial contaminants in the fermentation mix.

In embodiments, the effective concentration of inhibitor for use in methods provided herein can be determined empirically for a given production strain, contaminant strain, and production process. Alternatively, minimal data may be obtained for a given system and used to determine appropriate concentrations for inhibitors. Such determination is disclosed and demonstrated herein (see Examples), and is readily available to one of skill in the art, equipped with this disclosure.

Briefly, as described herein, growth and/or production competitiveness may be improved by, for example, i) adding a genetic trait that provides growth and/or production competitiveness, or ii) by providing growth conditions that increase growth and/or production competitiveness, e.g. through the addition of selective inhibitors. While not wishing to be bound by theory, in the first case, increased growth competitiveness of a strain A carrying a genetic trait that provides growth competitiveness will have a higher biomass ratio after a growth phase compared to a competing strain B than an strain C isogenic to strain A without the genetic trait, i.e. $cx_{A_mod}(t)/cx_{B_mod}(t)>cx_C(t)/cx_B(t)$. In the later case, strain A will have a higher biomass ratio after a growth phase compared to a competing strain B under the conditions promoting growth competitiveness, i.e. cx_A (t,c(inhibitor)>0 g/L)/cx_B (t,c(inhibitor)>0 g/L)>cx_A(t,c(inhibitor)=0 g/L)/cx_A(t, c(inhibitor)=0 g/L). After a growth phase indicates that the biomass of strain A had to increase during the respective time interval, i.e. $cx_A(t)>cx_A(t_{start})$. While not wishing to be bound by theory, in the first case, increased production competitiveness of a strain A carrying a genetic trait that provides production competitiveness will have a higher product ratio compared to a product of a competing strain B than an strain C isogenic to strain A without the genetic trait, i.e. $cp_A(t)/cp_B(t)>cp_C(t)/cp_B(t)$. In the later case, strain A will have a higher product ratio compared to a product of a competing strain B, i.e. cp_A (t,c(inhibitor)>0 g/L)/cp_B (t,c(inhibitor)>0 g/L)>cp_A(t,c(inhibitor)=0 g/L)/cp_B(t, c(inhibitor)=0 g/L).

Under situations where substrates are not limiting, e.g. under glucose excess conditions, maximum specific growth rate of the strains under the given cultivation conditions (medium, temperature, etc.) will be a component for determining growth competitiveness. Changes in the given conditions (e.g. changing concentrations of products, substrates, signaling molecules, etc.) may result in different values of maximum specific growth rate, and the maximum specific growth rate of strains may be different in a given condition. Considering such factors and assuming a constant μ_{max} for an exponential growth phase, the biomass concentration during the exponential growth phase that started at t_{lag} can be approximately described according to

$$c_X(t)=c_X(t)\cdot e^{\mu_{max}\cdot(t-t_{lag})} \quad \text{Eq. (1)}$$

Under aerobic, glucose-excess conditions and acetic acid in the medium, maximum specific growth rate of an exemplary ethanologen *S. cerevisiae* strain (PNY 827) was determined to be 0.61 l/h. In contrast, an isobutanologen *S. cerevisiae* strain (PNY 2129, constructed using PNY827) exhibited a maximum specific growth rate of 0.16 l/h. If an aerobic batch cultivation with a mixed culture consisting of both strains with a biomass ratio of 1:1, i.e. with a cell dry weight concentration of 1 g/L each, would be started, and both strains would be growing for 8 hours at μ_{max} without any lag phase, at the end of the process PNY 827 would account for approximately 131.6 g/L cell dry weight in the mixed culture, while PNY 2129 would account for only about 3.6 g/L. The ratio of biomass PNY 2129/PNY 827 would be below 3%. This phenomenon is illustrated in FIG. **18** where PNY 2129 is represented by strain A and PNY 827 by strain B.

In order to describe growth performance of a strain according to Eq. (1) under the influence of different inhibi-

tor/mixture of inhibitors concentrations in a batch experiment, μ_{max} was determined in a way to incorporate effect of the inhibitor/mixture of inhibitors. According to the observed inhibition kinetics, usually a "squared" inhibition term according to Eq. (2) was applied,

$$\mu_{max} = \frac{\mu^{o}_{max}}{\left(1 + \frac{c(I)^2}{K_I^2}\right)}, \quad \text{Eq. (2)}$$

with μ_{max} denoting a strain characteristic maximum specific growth rate at the inhibitor concentration c(I), and μ^{o}_{max} the maximum specific growth rate of the strain under the same conditions, but without inhibitor (c(inhibitor)=0 g/L). Finally K_I represents an inhibitory constant K_I.

In some occasions, inhibition of a cellular process significantly reduces but not completely abolishes growth of the organism. This effect can sometimes be explained by the action of alternative cellular processes available to the organism. In this particular situation, the "squared" inhibition term according to Eq. (2) is insufficient to describe growth of the strain, and a hybrid modeling approach according to Eq. (3) was used instead according to

$$\mu_{max} = \frac{\mu^{o}_{max\ 1}}{\left(1 + \frac{c(I)^2}{K_I^2}\right)} + \mu^{o}_{max\ 2}, \quad \text{Eq. (3)}$$

with μ_{max} denoting a strain characteristic maximum specific growth rate at the inhibitor concentration c(I), and the sum of μ^{o}_{max1} and μ^{o}_{max2} the maximum specific growth rate of the strain under the same conditions, but without inhibitor (c(inhibitor)=0 g/L). Finally K_I represents an inhibitory constant K_I. Using such equations to fit minimal data collected for a given system allows for determination of strain-specific parameters, i.e. of a maximum specific growth rate without inhibitor (μ^{o}_{max} or sum of μ^{o}_{max1} and μ^{o}_{max2}) and an inhibitory constant K_I. Based on these parameters, effect of inhibitor concentrations on maximum specific growth rate μ_{max} of a given production or contaminant strain can be made, as well as the IC50 value of the inhibitor on their growth be estimated. Equipped with this disclosure, one of skill in the art will be able to utilize parameters such as the IC50 to determine suitable concentrations of compounds for methods provided herein.

One embodiment is directed to a method for improving production competitiveness of a butanologen in a fermentation mix, wherein the method comprises contacting a genetically modified host cell and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, as well as a contaminating organism, and wherein the improved production competitiveness is associated with a higher butanol yield on the consumed substrate.

One embodiment is directed to a method for improving production competitiveness of a butanologen in a fermentation mix, wherein the method comprises contacting a genetically modified host cell and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, as well as an ethanologen yeast, and wherein the improved production competitiveness is associated with a higher butanol-to-ethanol ratio as compared to a cultivation without addition of one or more inhibitors, antibiotics, or combinations thereof.

One embodiment is directed to a method for improved production competitiveness of a butanologen in a fermentation mix, wherein the method comprises contacting a genetically modified host cell and a fermentation medium comprising one or more inhibitors, antibiotics, or combinations thereof, and wherein the addition of the one or more inhibitors, antibiotics, or combinations thereof results in less than a 20% loss in the yield of a lower alkyl alcohol produced by the host cell due to the presence of microbial contaminants. In some embodiments, the addition of the one or more inhibitors of amino acid synthesis, antibiotics, or combinations thereof results in less than a 10% loss in the yield of a lower alkyl alcohol produced by the host cell due to the presence of microbial contaminants.

It will be appreciated that compounds such as an inhibitor, antibiotic, or combinations thereof can be incorporated into a fermentation mix using any method known in the art. In embodiments, compounds are introduced by incorporation into a fermentation feed. In embodiments, compounds are introduced as a bolus or over the course of a fermentation process or a portion of the process as suitable for the compound and production process.

Alcohol Production

Disclosed herein are processes suitable for production of fermentation products from a carbon substrate. In one embodiment a lower alcohol is produced. In one embodiment, butanol is produced, and a butanologen is employed. In another embodiment, isobutanol is produced, and an isobutanologen is employed. In some embodiments, isobutanologens may comprise an isobutanol biosynthetic pathway, such as, but not limited to isobutanol biosynthetic pathways disclosed elsewhere herein. The ability to utilize carbon substrates to produce isobutanol can be confirmed using methods known in the art, including, but not limited to those described in U.S. Pat. Nos. 7,851,188 and 7,993,889 which are incorporated herein by reference. For example, to confirm isobutanol production, the concentration of isobutanol in the culture media can be determined by a number of methods known in the art. For example, a specific high performance liquid chromatography (HPLC) method utilized a Shodex SH-1011 column with a Shodex SH-G guard column, both purchased from Waters Corporation (Milford, Mass.), with refractive index (RI) detection. Chromatographic separation was achieved using 0.01 M H_2SO_4 as the mobile phase with a flow rate of 0.5 mL/min and a column temperature of 50° C. Isobutanol had a retention time of 46.6 min under the conditions used. Alternatively, gas chromatography (GC) methods are available. For example, a specific GC method utilized an HP-INNOWax column (30 m×0.53 mm id, 1 μm film thickness, Agilent Technologies, Wilmington, Del.), with a flame ionization detector (FID). The carrier gas was helium at a flow rate of 4.5 mL/min, measured at 150° C. with constant head pressure; injector split was 1:25 at 200° C.; oven temperature was 45° C. for 1 min, 45 to 220° C. at 10° C./min, and 220° C. for 5 min; and FID detection was employed at 240° C. with 26 mL/min helium makeup gas. The retention time of isobutanol was 4.5 min.

In some embodiments, the butanologen comprises an engineered butanol pathway. In some embodiments, the butanologen is an isobutanologen. In some embodiments, the butanologen is a yeast. In some embodiments, the butanologen is a member of a genus of *Saccharomyces, Schizosaccharomyces, Hansenula, Candida, Kluyveromyces, Yarrowia, Issatchenkia*, or *Pichia*. In some embodiments, the butanologen is *Saccharomyces cerevisiae*.

In some embodiments, the engineered isobutanologen contains one or more polypeptides selected from a group of enzymes having the following Enzyme Commission Numbers: EC 2.2.1.6, EC 1.1.1.86, EC 4.2.1.9, EC 4.1.1.72, EC 1.1.1.1, EC 1.1.1.265, EC 1.1.1.2, EC 1.2.4.4, EC 1.3.99.2, EC 1.2.1.57, EC 1.2.1.10, EC 2.6.1.66, EC 2.6.1.42, EC 1.4.1.9, EC 1.4.1.8, EC 4.1.1.14, EC 2.6.1.18, EC 2.3.1.9, EC 2.3.1.16, EC 1.1.130, EC 1.1.1.35, EC 1.1.1.157, EC 1.1.1.36, EC 4.2.1.17, EC 4.2.1.55, EC 1.3.1.44, EC 1.3.1.38, EC 5.4.99.13, EC 4.1.1.5, EC 2.7.1.29, EC 1.1.1.76, EC 1.2.1.57, and EC 4.2.1.28.

In some embodiments, the engineered isobutanologen contains one or more polypeptides selected from acetolactate synthase, acetohydroxy acid isomeroreductase, acetohydroxy acid dehydratase, branched-chain alpha-keto acid decarboxylase, branched-chain alcohol dehydrogenase, acylating aldehyde dehydrogenase, branched-chain keto acid dehydrogenase, butyryl-CoA dehydrogenase, transaminase, valine dehydrogenase, valine decarboxylase, omega transaminase, acetyl-CoA acetyltransferase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, isobutyryl-CoA mutase, acetolactate decarboxylase, acetonin aminase, butanol dehydrogenase, butyraldehyde dehydrogenase, acetoin kinase, acetoin phosphate aminase, aminobutanol phosphate phospholyase, aminobutanol kinase, butanediol dehydrogenase, and butanediol dehydratase.

In some embodiments, the carbon substrate is selected from the group consisting of: oligosaccharides, polysaccharides, monosaccharides, and mixtures thereof. In some embodiments, the carbon substrate is selected from the group consisting of: fructose, glucose, lactose, maltose, galactose, sucrose, starch, cellulose, feedstocks, ethanol, lactate, succinate, glycerol, corn mash, sugar cane, biomass, a C5 sugar, such as xylose and arabinose, and mixtures thereof.

In some embodiments, the engineered isobutanol pathway comprises the following substrate to product conversions:

a. pyruvate to acetolactate
b. acetolactate to 2,3-dihydroxyisovalerate
c. 2,3-dihydroxyisovalerate to α-ketoisovalerate
d. α-ketoisovalerate to isobutyraldehyde, and
e. isobutyraldehyde to isobutanol.

In some embodiments, one or more of the substrate to product conversions utilizes NADH or NADPH as a cofactor.

In some embodiments, enzymes from the biosynthetic pathway are localized to the cytosol. In some embodiments, enzymes from the biosynthetic pathway that are usually localized to the mitochondria are localized to the cytosol. In some embodiments, an enzyme from the biosynthetic pathway is localized to the cytosol by removing the mitochondrial targeting sequence. In some embodiments, mitochondrial targeting is eliminated by generating new start codons as described in e.g., U.S. Pat. Nos. 7,851,188 and 7,993,889, which are incorporated herein by reference in its entirety. In some embodiments, the enzyme from the biosynthetic pathway that is localized to the cytosol is DHAD. In some embodiments, the enzyme from the biosynthetic pathway that is localized to the cytosol is KARI.

In some embodiments, the butanologen produces butanol at least 90% of effective yield, at least 91% of effective yield, at least 92% of effective yield, at least 93% of effective yield, at least 94% of effective yield, at least 95% of effective yield, at least 96% of effective yield, at least 97% of effective yield, at least 98% of effective yield, or at least 99% of effective yield. In some embodiments, the butanologen produces butanol at least 55% to at least 75% of effective yield, at least 50% to at least 80% of effective yield, at least 45% to at least 85% of effective yield, at least 40% to at least 90% of effective yield, at least 35% to at least 95% of effective yield, at least 30% to at least 99% of effective yield, at least 25% to at least 99% of effective yield, at least 10% to at least 99% of effective yield or at least 10% to at least 100% of effective yield.

In some embodiments, the host cell produces ethanol at least 90% of effective yield, at least 91% of effective yield, at least 92% of effective yield, at least 93% of effective yield, at least 94% of effective yield, at least 95% of effective yield, at least 96% of effective yield, at least 97% of effective yield, at least 98% of effective yield, or at least 99% of effective yield. In some embodiments, the host cell produces ethanol at least 55% to at least 75% of effective yield, at least 50% to at least 80% of effective yield, at least 45% to at least 85% of effective yield, at least 40% to at least 90% of effective yield, at least 35% to at least 95% of effective yield, at least 30% to at least 99% of effective yield, at least 25% to at least 99% of effective yield, at least 10% to at least 99% of effective yield or at least 10% to at least 100% of effective yield.

In some embodiments, the host cell produces a C3-C6 alcohol at least 90% of effective yield, at least 91% of effective yield, at least 92% of effective yield, at least 93% of effective yield, at least 94% of effective yield, at least 95% of effective yield, at least 96% of effective yield, at least 97% of effective yield, at least 98% of effective yield, or at least 99% of effective yield. In some embodiments, the host cell produces a C3-C6 alcohol at least 55% to at least 75% of effective yield, at least 50% to at least 80% of effective yield, at least 45% to at least 85% of effective yield, at least 40% to at least 90% of effective yield, at least 35% to at least 95% of effective yield, at least 30% to at least 99% of effective yield, at least 25% to at least 99% of effective yield, at least 10% to at least 99% of effective yield or at least 10% to at least 100% of effective yield.

One embodiment of this invention is directed to a method for the production of a C3-C6 alcohol comprising:

a. providing a host cell with an engineered pyruvate-utilizing pathway and a polypeptide conferring resistance to one or more inhibitors, antibiotics or combinations thereof, wherein the engineered pyruvate-utilizing pathway is a C3-C6 alcohol biosynthetic pathway;
b. contacting the host cell with a fermentable carbon substrate in a fermentation medium under conditions whereby the C3-C6 alcohol is produced; and
c. recovering the C3-C6 alcohol.

In some embodiments, the fermentation medium comprises one or more inhibitors, antibiotics or combinations thereof.

In some embodiments, the C3-C6 alcohol is produced at a titer from about 5 g/L to about 100 g/L. In some embodiments, the C3-C6 alcohol is produced at a titer of at least 20 g/L. In some embodiments, the C3-C6 alcohol is selected from the group consisting of: butanol, isobutanol, propanol, and isopropanol.

One embodiment is a method for the production of ethanol comprising:

a. providing a host cell with a pyruvate-utilizing pathway and a polypeptide conferring resistance to one or more inhibitors, antibiotics or combinations thereof, wherein the pyruvate-utilizing pathway is an ethanol producing pathway;

b. contacting the host cell with a fermentable carbon substrate in a fermentation medium under conditions whereby the ethanol is produced; and
c. recovering the ethanol.

In some embodiments, the invention provides a method for production of a fermentation product in a fermentation process comprising contacting a fermentation mix comprising a recombinant production microorganism which comprises a pyruvate-utilizing pathway with at least one compound which preferentially inhibits at least one contaminant microorganism. In some embodiments the inhibition is measured through a reduction in the specific growth rate. In some embodiments the inhibition is measured through a reduced specific product formation rate of the contaminant. In some embodiments, the specific growth rate of the at least one contaminant microorganism is reduced more than the specific growth rate of the recombinant production microorganism. In some embodiments, the production of the fermentation product of the at least one contaminant microorganism is reduced more than production of the fermentation product of the recombinant production microorganism.

In some embodiments, the major product of a production microorganism is ethanol. In some embodiments, the titer of ethanol that is produced may be at least about 80 g/L to at least about 120 g/L. In some embodiments, the titer of ethanol that is produced is least about 50 g/L, at least about 60 g/L, at least about 70 g/L, at least about 80 g/L, at least about 90 g/L, at least about 95 g/L, at least about 100 g/L, at least about 105 g/L, at least about 110 g/L, at least about 115 g/L, or at least about 120 g/L.

In some embodiments, the major product of a production microorganism is butanol. In some embodiments, the titer of butanol that is produced may be at least about 80 g/L to at least about 120 g/L. In some embodiments, the titer of butanol that is produced is least about 50 g/L, at least about 60 g/L, at least about 70 g/L, at least about 80 g/L, at least about 90 g/L, at least about 95 g/L, at least about 100 g/L, at least about 105 g/L, at least about 110 g/L, at least about 115 g/L, or at least about 120 g/L.

In some embodiments, the major product of a production microorganism is butanol, for example, isobutanol. In some embodiments, the major product of a contaminant microorganism is ethanol. The titer of ethanol may be less than that of butanol. In some embodiments, the titer of ethanol is less than about 20 g/L, 10 g/L, less than about 5 g/L, or less than about 2 g/L.

In embodiments, the major product of a production microorganism is butanol, for example isobutanol, and the major product of a contaminant microorganism is ethanol. In embodiments, the percentage of ethanol produced as a fraction of the amount of butanol produced is less than about 25%, less than about 20%, less than about 10%, less than about 5%, less than about 2%, or less than about 1%.

In some embodiments, butanol is contacted with a fatty acid and a lipase producing a fatty acid butyl ester ("FABE"), which may be used as a biodiesel fuel.

The reduction in contamination can be measured through any assay known in the art, including, but not limited to, standard plating assays, qPCR/RT-PCR, or by measuring fermentation titer, yield, or specific growth rate of a production microorganism in relation to a contaminant microorganism.

In some embodiments, reduction in contamination and increased production competitiveness of the butanologen is observed through measurement of the ratio of the desired fermentation product to the contaminant fermentation production (e.g. butanol to ethanol). As the contaminant microorganism is inhibited to a greater degree than the production microorganism is inhibited, by either specific inhibitors or different concentrations of those inhibitors, the ratio of the desired fermentation product to the contaminant fermentation product will increase. The production of fermentation product in the aqueous phase can be quantified by HPLC, as described in the General Methods Section.

In some embodiments, the reduction in contamination is observed through measurement of the specific growth rate of samples treated with varying concentrations of inhibitors in a cell suspension, and measuring the OD of the samples at designated time points.

In some embodiments, the reduction in contamination is seen through the use of plating assays. In some embodiments, early stationary phase cultures are used to inoculate top agar media which is poured onto petri plates. Filter disks containing different concentrations of inhibitor can be applied to the plate surface, and, after a period of incubation, zones of growth inhibition can be observed.

Host Cells and Microorganisms

The terms "host cell" and "microorganism" are synonymous and used interchangeably throughout. In embodiments, suitable host cells include any yeast host useful for genetic modification and recombinant gene expression. In some embodiments, the host cell is a butanologen. In some embodiments, the host cell is an isobutanologen. In some embodiments, the isobutanologen host cell can be a member of the genera *Schizosaccharomyces, Issatchenkia, Kluyveromyces, Yarrowia, Pichia, Candida, Hansenula, Aspergillus, Pachysolen, Rhodotorula, Zygosaccharomyces, Galactomyces, Torulaspora, Debayomyces, Williopsis, Dekkera, Kloeckera, Metschnikowia* or *Saccharomyces*. In other embodiments, the host cell can be *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces thermotolerans, Kluyveromyces marxianus, Candida glabrata, Candida albicans, Pichia stipitis, Yarrowia lipolytica, E. coli*, or *L. plantarum*. In still other embodiments, the host cell is a yeast host cell. In some embodiments, the host cell is a member of the genera *Saccharomyces*. In some embodiments, the host cell is *Kluyveromyces lactis, Candida glabrata* or *Schizosaccharomyces pombe*. In some embodiments, the host cell is *Saccharomyces cerevisiae. S. cerevisiae* yeast are known in the art and are available from a variety of sources, including, but not limited to, American Type Culture Collection (Rockville, Md.), Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, LeSaffre, Gert Strand AB, Ferm Solutions, North American Bioproducts, Martrex, and Lallemand. *S. cerevisiae* include, but are not limited to, BY4741, CEN.PK 113-7D, Ethanol Red® yeast, Ferm Pro™ yeast, Bio-Ferm® XR yeast, Gert Strand Prestige Batch Turbo alcohol yeast, Gert Strand Pot Distillers yeast, Gert Strand Distillers Turbo yeast, FerMax™ Green yeast, FerMax™ Gold yeast, Thermosacc® yeast, BG-1, PE-2, CAT-1, CBS7959, CBS7960, and CBS7961.

Saccharomyces cerevisiae PNY860 (or PNY0860), described in Example 4, was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va., 20110 on Jul. 21, 2011, and assigned ATCC Accession No. PTA-12007.

Saccharomyces cerevisiae PNY827, described in Examples 3 and 13, was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va., 20110 on Sep. 22, 2011, and assigned ATCC Accession No. PTA-12105.

In some embodiments, the host cell expresses an engineered butanol biosynthetic pathway. From time to time,

such a host cell is referred to as a "butanologen". In some embodiments, the butanologen is an isobutanologen expressing an engineered isobutanol biosynthetic pathway. In some embodiments, the butanologen is a bacteria, cyanobacteria or filamentous fungi. In some embodiments, the genus of the host cell is selected from the group consisting of: *Salmonella, Arthrobacter, Bacillus, Brevibacterium, Clostridium, Corynebacterium, Gluconobacter, Nocardia, Pseudomonas, Rhodococcus, Streptomyces, Zymomonas, Escherichia, Lactobacillus, Lactococcus, Enterococcus, Alcaligenes, Klebsiella, Paenibacillus*, and *Xanthomonas*.

Some embodiments comprise a genetically modified host cell comprising:

a. an engineered C3-C6 alcohol biosynthetic pathway; and,

b. a polypeptide that is resistant to inhibitors, antibiotics, or a combination thereof.

Carbon Substrates

Suitable carbon substrates may include, but are not limited to, monosaccharides such as fructose or glucose, oligosaccharides such as lactose, maltose, galactose, or sucrose, polysaccharides such as starch or cellulose or mixtures thereof and unpurified mixtures from renewable feedstocks such as cheese whey permeate, cornsteep liquor, sugar beet molasses, and barley malt. Other carbon substrates may include ethanol, lactate, succinate, or glycerol.

"Sugar" includes monosaccharides such as fructose or glucose, oligosaccharides such as lactose, maltose, galactose, or sucrose, polysaccharides such as starch or cellulose, C5 sugars such as xylose and arabinose, and mixtures thereof.

Additionally the carbon substrate may also be one-carbon substrates such as carbon dioxide, or methanol for which metabolic conversion into key biochemical intermediates has been demonstrated. In addition to one and two carbon substrates, methylotrophic organisms are also known to utilize a number of other carbon containing compounds such as methylamine, glucosamine and a variety of amino acids for metabolic activity. For example, methylotrophic yeasts are known to utilize the carbon from methylamine to form trehalose or glycerol (Bellion et al., *Microb. Growth C1 Compd., [Int. Symp.]*, 7th (1993), 415-32, Editor(s): Murrell, J. Collin; Kelly, Don P. Publisher: Intercept, Andover, UK). Similarly, various species of *Candida* will metabolize alanine or oleic acid (Sulter et al., *Arch. Microbiol.* 153:485-489 (1990)). Hence it is contemplated that the source of carbon utilized in the present invention may encompass a wide variety of carbon containing substrates and will only be limited by the choice of organism.

Although it is contemplated that all of the above mentioned carbon substrates and mixtures thereof are suitable in the present invention, in some embodiments, the carbon substrates are glucose, fructose, and sucrose, or mixtures of these with C5 sugars such as xylose and arabinose for yeasts cells modified to use C5 sugars. Sucrose may be derived from renewable sugar sources such as sugar cane, sugar beets, cassava, sweet sorghum, and mixtures thereof. Glucose and dextrose may be derived from renewable grain sources through saccharification of starch based feedstocks including grains such as corn, wheat, rye, barley, oats, and mixtures thereof. In addition, fermentable sugars may be derived from renewable cellulosic or lignocellulosic biomass through processes of pretreatment and saccharification, as described, for example, in U.S. Patent Application Publication No. 20070031918 A1, which is incorporated herein by reference. Biomass includes materials comprising cellulose, and optionally further comprising hemicellulose, lignin, starch, oligosaccharides and/or monosaccharides. Biomass may also comprise additional components, such as protein and/or lipid. Biomass may be derived from a single source, or biomass can comprise a mixture derived from more than one source; for example, biomass may comprise a mixture of corn cobs and corn stover, or a mixture of grass and leaves. Biomass includes, but is not limited to, bioenergy crops, agricultural residues, municipal solid waste, industrial solid waste, sludge from paper manufacture, yard waste, wood and forestry waste. Examples of biomass include, but are not limited to, corn grain, corn cobs, crop residues such as corn husks, corn stover, grasses, wheat, wheat straw, barley, barley straw, hay, rice straw, switchgrass, waste paper, sugar cane bagasse, sorghum, soy, components obtained from milling of grains, trees, branches, roots, leaves, wood chips, sawdust, shrubs and bushes, vegetables, fruits, flowers, animal manure, and mixtures thereof.

In some embodiments, the carbon substrate is glucose derived from corn. In some embodiments, the carbon substrate is glucose derived from wheat. In some embodiments, the carbon substrate is sucrose derived from sugar cane.

In addition to an appropriate carbon source, fermentation media must contain suitable minerals, salts, cofactors, buffers and other components, known to those skilled in the art, suitable for the growth of the cultures and promotion of an enzymatic pathway described herein.

Fermentation Conditions

Typically cells are grown at a temperature in the range of about 20° C. to about 40° C. in an appropriate medium. Suitable growth media in the present invention include common commercially prepared media such as Sabouraud Dextrose (SD) broth, Yeast Medium (YM) broth, or broth that includes yeast nitrogen base, ammonium sulfate, and dextrose (as the carbon/energy source) or YPD Medium, a blend of peptone, yeast extract, and dextrose in optimal proportions for growing most *Saccharomyces cerevisiae* strains. Other defined or synthetic growth media may also be used, and the appropriate medium for growth of the particular microorganism will be known by one skilled in the art of microbiology or fermentation science. The use of agents known to modulate catabolite repression directly or indirectly, e.g., cyclic adenosine 2':3'-monophosphate, may also be incorporated into the fermentation medium.

Suitable pH ranges for the fermentation are from about pH 5.0 to about pH 9.0. In one embodiment, about pH 6.0 to about pH 8.0 is used for the initial condition. Suitable pH ranges for the fermentation of yeast are typically from about pH 3.0 to about pH 9.0. In one embodiment, about pH 5.0 to about pH 8.0 is used for the initial condition. Suitable pH ranges for the fermentation of other microorganisms are from about pH 3.0 to about pH 7.5. In one embodiment, about pH 4.5 to about pH 6.5 is used for the initial condition.

Fermentations may be performed under aerobic or anaerobic conditions. In one embodiment, anaerobic or microaerobic conditions are used for fermentations.

Industrial Batch and Continuous Fermentations

Isobutanol, or other products, may be produced using a batch method of fermentation. A classical batch fermentation is a closed system where the composition of the medium is set at the beginning of the fermentation and not subject to artificial alterations during the fermentation. A variation on the standard batch system is the fed-batch system. Fed-batch fermentation processes are also suitable in the present invention and comprise a typical batch system with the exception that the substrate is added in increments as the fermentation progresses. Fed-batch systems are useful when catabolite repression is apt to inhibit the metabolism of the cells and

where it is desirable to have limited amounts of substrate in the media. Batch and fed-batch fermentations are common and well known in the art and examples may be found in Thomas D. Brock in *Biotechnology: A Textbook of Industrial Microbiology*, Second Edition (1989) Sinauer Associates, Inc., Sunderland, Mass., or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.,* 36:227, (1992).

Isobutanol, or other products, may also be produced using continuous fermentation methods. Continuous fermentation is an open system where a defined fermentation medium is added continuously to a bioreactor and an equal amount of conditioned media is removed simultaneously for processing. Continuous fermentation generally maintains the cultures at a constant high density where cells are primarily in log phase growth. Continuous fermentation allows for the modulation of one factor or any number of factors that affect cell growth or end product concentration. Methods of modulating nutrients and growth factors for continuous fermentation processes as well as techniques for maximizing the rate of product formation are well known in the art of industrial microbiology and a variety of methods are detailed by Brock, supra.

It is contemplated that the production of isobutanol, or other products, may be practiced using batch, fed-batch or continuous processes and that any known mode of fermentation would be suitable. Additionally, it is contemplated that cells may be immobilized on a substrate as whole cell catalysts or encapsulated within porous material (e.g. alginate beads) and subjected to fermentation conditions for isobutanol production.

Methods for Isobutanol Isolation from the Fermentation Medium

Bioproduced isobutanol may be isolated from the fermentation medium using methods known in the art for ABE fermentations (see, e.g., Durre, *Appl. Microbiol. Biotechnol.* 49:639-648 (1998), Groot et al., *Process. Biochem.* 27:61-75 (1992), and references therein). For example, solids may be removed from the fermentation medium by centrifugation, filtration, decantation, or the like. Then, the isobutanol may be isolated from the fermentation medium using methods such as distillation, azeotropic distillation, liquid-liquid extraction, adsorption, gas stripping, membrane evaporation, or pervaporation.

Because isobutanol forms a low boiling point, azeotropic mixture with water, distillation can be used to separate the mixture up to its azeotropic composition. Distillation may be used in combination with another separation method to obtain separation around the azeotrope. Methods that may be used in combination with distillation to isolate and purify butanol include, but are not limited to, decantation, liquid-liquid extraction, adsorption, and membrane-based techniques. Additionally, butanol may be isolated using azeotropic distillation using an entrainer (see, e.g., Doherty and Malone, *Conceptual Design of Distillation Systems*, McGraw Hill, New York, 2001).

The butanol-water mixture forms a heterogeneous azeotrope so that distillation may be used in combination with decantation to isolate and purify the isobutanol. In this method, the isobutanol containing fermentation broth is distilled to near the azeotropic composition. Then, the azeotropic mixture is condensed, and the isobutanol is separated from the fermentation medium by decantation. The decanted aqueous phase may be returned to the first distillation column as reflux. The isobutanol-rich decanted organic phase may be further purified by distillation in a second distillation column.

The isobutanol can also be isolated from the fermentation medium using liquid-liquid extraction in combination with distillation. In this method, the isobutanol is extracted from the fermentation broth using liquid-liquid extraction with a suitable solvent. The isobutanol-containing organic phase is then distilled to separate the butanol from the solvent.

Distillation in combination with adsorption can also be used to isolate isobutanol from the fermentation medium. In this method, the fermentation broth containing the isobutanol is distilled to near the azeotropic composition and then the remaining water is removed by use of an adsorbent, such as molecular sieves (Aden et al., *Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover*, Report NREL/TP-510-32438, National Renewable Energy Laboratory, June 2002).

Additionally, distillation in combination with pervaporation may be used to isolate and purify the isobutanol from the fermentation medium. In this method, the fermentation broth containing the isobutanol is distilled to near the azeotropic composition, and then the remaining water is removed by pervaporation through a hydrophilic membrane (Guo et al., *J. Membr. Sci.* 245, 199-210 (2004)).

In situ product removal (ISPR) (also referred to as extractive fermentation) can be used to remove butanol (or other fermentative alcohol) from the fermentation vessel as it is produced, thereby allowing the microorganism to produce butanol at high yields. One method for ISPR for removing fermentative alcohol that has been described in the art is liquid-liquid extraction. In general, with regard to butanol fermentation, for example, the fermentation medium, which includes the microorganism, is contacted with an organic extractant at a time before the butanol concentration reaches a toxic level. The organic extractant and the fermentation medium form a biphasic mixture. The butanol partitions into the organic extractant phase, decreasing the concentration in the aqueous phase containing the microorganism, thereby limiting the exposure of the microorganism to the inhibitory butanol.

Liquid-liquid extraction can be performed, for example, according to the processes described in U.S. Patent Appl. Pub. No. 2009/0305370, the disclosure of which is hereby incorporated in its entirety. U.S. Patent Appl. Pub. No. 2009/0305370 describes methods for producing and recovering butanol from a fermentation broth using liquid-liquid extraction, the methods comprising the step of contacting the fermentation broth with a water immiscible extractant to form a two-phase mixture comprising an aqueous phase and an organic phase. Typically, the extractant can be an organic extractant selected from the group consisting of saturated, mono-unsaturated, poly-unsaturated (and mixtures thereof) C_{12} to C_{22} fatty alcohols, C_{12} to C_{22} fatty acids, esters of C_{12} to C_{22} fatty acids, C_{12} to C_{22} fatty aldehydes, and mixtures thereof. The extractant(s) for ISPR can be non-alcohol extractants. The ISPR extractant can be an exogenous organic extractant such as oleyl alcohol, behenyl alcohol, cetyl alcohol, lauryl alcohol, myristyl alcohol, stearyl alcohol, 1-undecanol, oleic acid, lauric acid, myristic acid, stearic acid, methyl myristate, methyl oleate, undecanal, lauric aldehyde, 20-methylundecanal, and mixtures thereof.

In some embodiments, an ester can be formed by contacting the alcohol in a fermentation medium with an organic acid (e.g., fatty acids) and a catalyst capable of esterfiying the alcohol with the organic acid. In such embodiments, the organic acid can serve as an ISPR extractant into which the alcohol esters partition. The organic acid can be supplied to the fermentation vessel and/or derived from the biomass

supplying fermentable carbon fed to the fermentation vessel. Lipids present in the feedstock can be catalytically hydrolyzed to organic acid, and the same catalyst (e.g., enzymes) can esterify the organic acid with the alcohol. The catalyst can be supplied to the feedstock prior to fermentation, or can be supplied to the fermentation vessel before or contemporaneously with the supplying of the feedstock. When the catalyst is supplied to the fermentation vessel, alcohol esters can be obtained by hydrolysis of the lipids into organic acid and substantially simultaneous esterification of the organic acid with butanol present in the fermentation vessel. Organic acid and/or native oil not derived from the feedstock can also be fed to the fermentation vessel, with the native oil being hydrolyzed into organic acid. Any organic acid not esterified with the alcohol can serve as part of the ISPR extractant. The extractant containing alcohol esters can be separated from the fermentation medium, and the alcohol can be recovered from the extractant. The extractant can be recycled to the fermentation vessel. Thus, in the case of butanol production, for example, the conversion of the butanol to an ester reduces the free butanol concentration in the fermentation medium, shielding the microorganism from the toxic effect of increasing butanol concentration. In addition, unfractionated grain can be used as feedstock without separation of lipids therein, since the lipids can be catalytically hydrolyzed to organic acid, thereby decreasing the rate of build-up of lipids in the ISPR extractant. Other butanol product recovery and/or ISPR methods may be employed, including those described in U.S. Pat. No. 8,101,808, incorporated herein by reference.

In situ product removal can be carried out in a batch mode or a continuous mode. In a continuous mode of in situ product removal, product is continually removed from the reactor. In a batchwise mode of in situ product removal, a volume of organic extractant is added to the fermentation vessel and the extractant is not removed during the process. For in situ product removal, the organic extractant can contact the fermentation medium at the start of the fermentation forming a biphasic fermentation medium. Alternatively, the organic extractant can contact the fermentation medium after the microorganism has achieved a desired amount of growth, which can be determined by measuring the optical density of the culture. Further, the organic extractant can contact the fermentation medium at a time at which the product alcohol level in the fermentation medium reaches a preselected level. In the case of butanol production according to some embodiments of the present invention, the organic acid extractant can contact the fermentation medium at a time before the butanol concentration reaches a toxic level, so as to esterify the butanol with the organic acid to produce butanol esters and consequently reduce the concentration of butanol in the fermentation vessel. The ester-containing organic phase can then be removed from the fermentation vessel (and separated from the fermentation broth which constitutes the aqueous phase) after a desired effective titer of the butanol esters is achieved. In some embodiments, the ester-containing organic phase is separated from the aqueous phase after fermentation of the available fermentable sugar in the fermentation vessel is substantially complete.

Isobutanol titer in any phase can be determined by methods known in the art, such as via high performance liquid chromatography (HPLC) or gas chromatography, as described, for example in U.S. Patent Appl. Pub. No. US20090305370, which is incorporated herein by reference.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

General Methods

Standard recombinant DNA and molecular cloning techniques used in the Examples are well known in the art and are described by Sambrook et al. (Sambrook, J., Fritsch, E. F. and Maniatis, T. (Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989, here in referred to as Maniatis) and by Ausubel et al. (Ausubel et al., Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience, 1987).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General Bacteriology (Phillipp et al., eds., American Society for Microbiology, Washington, D.C., 1994) or by Thomas D. Brock in (Brock, Biotechnology: A Textbook of Industrial Microbiology, Second Edition, Sinauer Associates, Inc., Sunderland, Mass. (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of bacterial cells were obtained from Sigma-Aldrich Chemicals (St. Louis, Mo.), BD Diagnostic Systems (Sparks, Md.), Invitrogen (Carlsbad, Calif.), HiMedia (Mumbai, India), SD Fine chemicals (India), or Takara Bio Inc. (Shiga, Japan), unless otherwise specified.

The meaning of abbreviations is as follows: "sec" means second(s), "min" means minute(s), "h" means hour(s), "nm" means nanometers, "uL" or "μl" means microliter(s), "mL" means milliliter(s), "mg/mL" means milligram per milliliter, "L" means liter(s), "nm" means nanometers, "mM" means millimolar, "M" means molar, "mmol" means millimole(s), "μmole" means micromole(s), "kg" means kilogram, "g" means gram(s), "μg" means microgram(s) and "ng" means nanogram(s), "PCR" means polymerase chain reaction, "OD" means optical density, "OD600" means the optical density measured at a wavelength of 600 nm, "kDa" means kilodaltons, "g" can also mean the gravitation constant, "bp" means base pair(s), "kbp" means kilobase pair(s), "kb" means kilobase, "%" means percent, "% w/v" means weight/volume percent, "% v/v" means volume/volume percent, "HPLC" means high performance liquid chromatography, "g/L" means gram per liter, "μg/L" means microgram per liter, "ng/μL" means nanogram per microliter, "pmol/μL" means picomol per microliter, "RPM" means rotation per minute, "μmol/min/mg" means micromole per minute per milligram, "w/v" means weight per volume, "v/v" means volume per volume.

Example 1

Construction of Expression Vectors for Isobutanol Pathway Gene Expression in *S. cerevisiae*

pLH475-JEA1 Construction

The pLH475-JEA1 plasmid (SEQ ID NO:23) was constructed for expression of ALS and KARI in yeast. pLH475-JEA1 is a pHR81 vector (ATCC #87541) containing the following chimeric genes: (1) the CUP1 promoter (SEQ ID NO:24), acetolactate synthase coding region from *Bacillus subtilis* (AlsS; SEQ ID NO:25; protein SEQ ID NO:36) and

CYC1 terminator2 (SEQ ID NO:27); (2) an ILV5 promoter (SEQ ID NO:28), Pf5.IlvC-JEA1 coding region (SEQ ID NO:29; protein SEQ ID NO:30 and ILV5 terminator (SEQ ID NO:31); and (3) the FBA1 promoter (SEQ ID NO:32), *S. cerevisiae* KARI coding region (ILV5; SEQ ID NO:33; protein SEQ ID NO:34) and CYC1 terminator (SEQ ID NO:35). The Pf5.IlvC-JEA1 coding region is a sequence encoding KARI derived from *Pseudomonas fluorescens* but containing mutations, that was described in commonly owned and co-pending US Patent Application Publication US20100197519A1, which is herein incorporated by reference (Pf5.IlvC-JEA1 encoded KARI: SEQ ID NO:29; protein SEQ ID NO:30)

Expression Vector pLH468

The pLH468 plasmid (SEQ ID NO:39) was constructed for expression of DHAD, KivD and HADH in yeast. Coding regions for *L. lactis* ketoisovalerate decarboxylase (KivD) (SEQ ID NO:141) and Horse liver alcohol dehydrogenase (HADH) (SEQ ID NO:40 and 142) were synthesized by DNA2.0 based on codons that were optimized for expression in *Saccharomyces cerevisiae* and provided in plasmids pKivDy-DNA2.0 and pHadhy-DNA2.0. Individual expression vectors for KivD and HADH were constructed. To assemble pLH467 (pRS426::P_{TDH3}-kivDy-TDH3t), vector pNY8 (SEQ ID NO:14; also named pRS426.GPD-ald-GPDt, described in commonly owned and co-pending US Patent App. Pub. US2008/0182308, Example 17, which is herein incorporated by reference) was digested with AscI and SfiI enzymes, thus excising the GPD promoter and the ald coding region. A TDH3 promoter fragment (SEQ ID NO:41) from pNY8 was PCR amplified to add an AscI site at the 5' end, and an SpeI site at the 3' end, using 5' primer OT1068 and 3' primer OT1067 (SEQ ID NOs:42 and 43). The AscI/SfiI digested pNY8 vector fragment was ligated with the TDH3 promoter PCR product digested with AscI and SpeI, and the Spa-SfiI fragment containing the codon optimized kivD coding region isolated from the vector pKivD-DNA2.0. The triple ligation generated vector pLH467 (pRS426::P_{TDH3}-kivDy-TDH3t). pLH467 (SEQ ID NO:44) was verified by restriction mapping and sequencing.

pLH435 (pRS425::P_{GPM1}-Hadhy-ADH1t) (SEQ ID NO:52) was derived from vector pRS425::GPM-sadB (SEQ ID NO:45) which is described in commonly owned and co-pending US Patent App. Pub No. US20090305363 A1, Example 3, which is herein incorporated by reference in its entirety. pRS425::GPM-sadB is the pRS425 vector (ATCC #77106) with a chimeric gene containing the GPM1 promoter (SEQ ID NO: 46), coding region from a butanol dehydrogenase of *Achromobacter xylosoxidans* (sadB; DNA SEQ ID NO:47; protein SEQ ID NO:48: disclosed in U.S. Pat. No. 8,188,250, which is herein incorporated by reference in its entirety), and ADH1 terminator (SEQ ID NO:49). pRS425::GPMp-sadB contains BbvI and PacI sites at the 5' and 3' ends of the sadB coding region, respectively. A Nha site was added at the 5' end of the sadB coding region by site-directed mutagenesis using primers OT1074 and OT1075 (SEQ ID NO:50 and 51) to generate vector pRS425-GPMp-sadB-NheI, which was verified by sequencing. pRS425::P_{GPM1}-sadB-NheI was digested with NheI and PacI to drop out the sadB coding region, and ligated with the NheI-PacI fragment containing the codon optimized HADH coding region from vector pHadhy-DNA2.0 to create pLH435.

To combine KivD and HADH expression cassettes in a single vector, yeast vector pRS411 (ATCC #87474) was digested with Sad and Not I, and ligated with the SacI-SalI fragment from pLH467 that contains the P_{TDH3}-kivDy-TDH3t cassette together with the SalI-NotI fragment from pLH435 that contains the P_{GPM1}-Hadhy-ADH1t cassette in a triple ligation reaction. This yielded the vector pRS411::P_{TDH3}-kivDy-P_{GPM1}-Hadhy (pLH441), which was verified by restriction mapping.

In order to generate a co-expression vector for all three genes in the lower isobutanol pathway: ilvD, kivDy and Hadhy, we used pRS423 FBA ilvD(Strep) (SEQ ID NO:53, which is described in commonly owned and co-pending US Patent App. Pub. US 20100081154A1, which is herein incorporated by reference in its entirety, as the source of the IlvD gene. This shuttle vector contains an F1 origin of replication (nt 1423 to 1879) for maintenance in *E. coli* and a 2 micron origin (nt 8082 to 9426) for replication in yeast. The vector has an FBA1 promoter (nt 2111 to 3108; SEQ ID NO:32) and FBA terminator (nt 4861 to 5860; SEQ ID NO:54). In addition, it carries the His marker (nt 504 to 1163) for selection in yeast and ampicillin resistance marker (nt 7092 to 7949) for selection in *E. coli*. The ilvD coding region (nt 3116 to 4828; SEQ ID NO:55; protein SEQ ID NO:56) from *Streptococcus mutans* UA159 (ATCC #700610) is between the FBA promoter and FBA terminator forming a chimeric gene for expression. In addition there is a lumio tag fused to the ilvD coding region (nt 4829-4849).

The first step was to linearize pRS423 FBA ilvD(Strep) (also called pRS423-FBA(SpeI)-IlvD(*Streptococcus mutans*)-Lumio) with SacI and SacII (with SacII site blunt ended using T4 DNA polymerase), to give a vector with total length of 9,482 bp. The second step was to isolate the kivDy-hADHy cassette from pLH441 with SacI and KpaI (with KpaI site blunt ended using T4 DNA polymerase), which gives a 6,063 bp fragment. This fragment was ligated with the 9,482 bp vector fragment from pRS423-FBA (SpeI)-IlvD(*Streptococcus mutans*)-Lumio. This generated vector pLH468 (pRS423::P_{FBA1}-ilvD(Strep)Lumio-FBA1t-P_{TDH3}-kivDy-TDH3t-P_{GPM1}-hadhy-ADH1t), which was confirmed by restriction mapping and sequencing.

Example 2

Construction of *S. cerevisiae* Host Strain Containing Disruptions in Pyruvate Decarboxylase and Hexokinase II

This example describes insertion-inactivation of endogenous PDC1, PDC5, and PDC6 genes of *S. cerevisiae*. PDC1, PDC5, and PDC6 genes encode the three major isozymes of pyruvate decarboxylase. Hexokinase II, which is responsible for phosphorylation of glucose and transcriptional repression, is also inactivated. The resulting PDC/HXK2 inactivation strain (U.S. Publication No: 2011/0124060, which is incorporated herein by reference) was used as a host for expression vectors pLH475-JEA1 and pLH468 that were described in Example 2.

Construction of pdc6::P_{GPM1}-sadB Integration Cassette and PDC6 Deletion:

A pdc6::P_{GPM1}-sadB-ADH1t-URA3r integration cassette was made by joining the GPM-sadB-ADHt segment from pRS425::GPM-sadB (described above) to the URA3r gene from pUC19-URA3r. pUC19-URA3r (SEQ ID NO:57) contains the URA3 marker from pRS426 (ATCC #77107) flanked by 75 bp homologous repeat sequences to allow homologous recombination in vivo and removal of the URA3 marker. The two DNA segments were joined by SOE PCR (as described by Horton et al. (1989) *Gene* 77:61-68) using as template pRS425::GPM-sadB and pUC19-URA3r plasmid DNAs, with Phusion DNA polymerase (New Eng-

land Biolabs Inc., Beverly, Mass.; catalog no. F-5405) and primers 114117-11A through 114117-11D (SEQ ID NOs:58, 59, 60 and 61), and 114117-13A and 114117-13B (SEQ ID NOs:62 and 63).

The outer primers for the SOE PCR (114117-13A and 114117-13B) contained 5' and 3' ~50 bp regions homologous to regions upstream and downstream of the PDC6 promoter and terminator, respectively. The completed cassette PCR fragment was transformed into BY4700 (ATCC #200866) and transformants were maintained on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202). Transformants were screened by PCR using primers 112590-34G and 112590-34H (SEQ ID NOs:64 and 65), and 112590-34F and 112590-49E (SEQ ID NOs:66 and 67) to verify integration at the PDC6 locus with deletion of the PDC6 coding region. The URA3r marker was recycled by plating on synthetic complete media supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA media to verify the absence of growth. The resulting identified strain has the genotype: BY4700 pdc6::P_{GPM1}-sadB-ADH1t.

Construction of pdc1::P_{PDC1}-ilvD Integration Cassette and PDC1 Deletion:

A pdc1::P_{PDC1}-ilvD-FBA1t-URA3r integration cassette was made by joining the ilvD-FBA1t segment from pLH468 (described above) to the URA3r gene from pUC19-URA3r by SOE PCR (as described by Horton et al. (1989) Gene 77:61-68) using as template pLH468 and pUC19-URA3r plasmid DNAs, with Phusion DNA polymerase (New England Biolabs Inc., Beverly, Mass.; catalog no. F-540S) and primers 114117-27A through 114117-27D (SEQ ID NOs:68, 69, 70 and 71).

The outer primers for the SOE PCR (114117-27A and 114117-27D) contained 5' and 3' ~50 bp regions homologous to regions downstream of the PDC1 promoter and downstream of the PDC1 coding sequence. The completed cassette PCR fragment was transformed into BY4700 pdc6::P_{GPM1}-sadB-ADH1t and transformants were maintained on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202). Transformants were screened by PCR using primers 114117-36D and 135 (SEQ ID NOs:72 and 73), and primers 112590-49E and 112590-30F (SEQ ID NOs:67 and 74) to verify integration at the PDC1 locus with deletion of the PDC1 coding sequence. The URA3r marker was recycled by plating on synthetic complete media supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA media to verify the absence of growth. The resulting identified strain "NYLA67" has the genotype: BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t.

HIS3 Deletion

To delete the endogenous HIS3 coding region, a his3::URA3r2 cassette was PCR-amplified from URA3r2 template DNA (SEQ ID NO:75). URA3r2 contains the URA3 marker from pRS426 (ATCC #77107) flanked by 500 bp homologous repeat sequences to allow homologous recombination in vivo and removal of the URA3 marker. PCR was done using Phusion DNA polymerase and primers 114117-45A and 114117-45B (SEQ ID NOs:76 and 77) which generated a ~2.3 kb PCR product. The HIS3 portion of each primer was derived from the 5' region upstream of the HIS3 promoter and 3' region downstream of the coding region such that integration of the URA3r2 marker results in replacement of the HIS3 coding region. The PCR product was transformed into NYLA67 using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202) and transformants were selected on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. Transformants were screened to verify correct integration by replica plating of transformants onto synthetic complete media lacking histidine and supplemented with 2% glucose at 30° C. The URA3r marker was recycled by plating on synthetic complete media supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA media to verify the absence of growth. The resulting identified strain, called NYLA73, has the genotype: BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::Pp$_{PDC1}$-ilvD-FBA1t dhis3.

Deletion of HXK2 (Hexokinase II):

A hxk2::URA3r cassette was PCR-amplified from URA3r2 template (described above) using Phusion DNA polymerase and primers 384 and 385 (SEQ ID NOs:78 and 79) which generated a ~2.3 kb PCR product. The HXK2 portion of each primer was derived from the 5' region upstream of the HXK2 promoter and 3' region downstream of the coding region such that integration of the URA3r2 marker results in replacement of the HXK2 coding region. The PCR product was transformed into NYLA73 using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202) and transformants were selected on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. Transformants were screened by PCR to verify correct integration at the HXK2 locus with replacement of the HXK2 coding region using primers N869 and N871 (SEQ ID NOs:80 and 81). The URA3r2 marker was recycled by plating on synthetic complete media supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA media to verify the absence of growth, and by PCR to verify correct marker removal using primers N946 and N947 (SEQ ID NOs:82 and 83). The resulting identified strain named NYLA83 has the genotype: BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t dhis3 dhxk2.

Construction of pdc5::kanMX Integration Cassette and PDC5 Deletion

A pdc5::kanMX4 cassette was PCR-amplified from strain YLR134W chromosomal DNA (ATCC No. 4034091) using Phusion DNA polymerase and primers PDC5::KanMXF and PDC5::KanMXR (SEQ ID NOs:84 and 85) which generated a ~2.2 kb PCR product. The PDC5 portion of each primer was derived from the 5' region upstream of the PDC5 promoter and 3' region downstream of the coding region such that integration of the kanMX4 marker results in replacement of the PDC5 coding region. The PCR product was transformed into NYLA83, and transformants were selected and screened as described above. The identified correct transformants named NYLA84 have the genotype: BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t dhis3 dhxk2 pdc5::kanMX4.

Plasmid vectors pLH468 and pLH475-JEA1 were simultaneously transformed into strain NYLA84 (BY4700 pdc6::P_{GPM1}-sadB-ADH1t pdc1::P_{PDC1}-ilvD-FBA1t dhis3 dhxk2

pdc5::kanMX4) using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) and the resulting strain was maintained on synthetic complete media lacking histidine and uracil, and supplemented with 1% ethanol at 30° C.

Example 3

Construction of *S. cerevisiae* Host Strain Containing Disruptions in URA3, HIS3, and Insertion of Sulfonylurea-Resistant ILV2

This example describes inactivation of the URA3 and HIS3 genes of *S. cerevisiae*, and replacement of the native ILV2 gene with a variant that is resistant to sulfonylurea herbicides. The resulting strain will be used as a host for expression vectors pLH475-JEA1 and pLH468 that were described in Example 1.

URA3 Deletion

To delete the endogenous URA3 coding region, a deletion cassette was PCR-amplified from pLA54 (SEQ ID NO:100) which contains a P_{TEF1}-kanMX-TEF1t cassette flanked by loxP sites to allow homologous recombination in vivo and subsequent removal of the KanMX marker. PCR was performed using Phusion DNA polymerase and primers BK505 and BK506 (SEQ ID NOs:101 and 102). The URA3 portion of each primer was derived from the 5' region 180 bp upstream of the URA3 ATG and 3' region 78 bp downstream of the coding region such that integration of the KanMX cassette results in replacement of the URA3 coding region. The PCR product was transformed into PNY827 (ATCC # PTA-12105), using standard genetic techniques (Methods in Yeast Genetics, 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202) and transformants were selected on rich media supplemented 2% glucose and 100 μg/ml Geneticin at 30° C. Transformants were screened by colony PCR with primers BK468 and LA492 (SEQ ID NOs:103 and 104) to verify presence of the integration cassette. A heterozygous URA3 mutant was obtained; NYLA98 MATa/α URA3/ura3::loxP-kanMX-loxP. To obtain haploids, NYLA98 was sporulated using standard methods (Codón AC, Gasent-Ramírez J M, Benítez T., *Appl Environ Microbiol.* 1995 PMID: 7574601). Tetrads were dissected using a micromanipulator and grown on rich media supplemented with 2% glucose. Tetrads containing four viable spores were patched onto synthetic complete media lacking uracil and supplemented with 2% glucose, and the mating type was verified by multiplex colony PCR using primers AK109-1, AK109-2, and AK109-3 (SEQ ID NOs:105, 106, and 107). The resulting identified haploid strain called NYLA103 has the genotype MATα ura3Δ::loxP-kanMX-loxP, and NYLA106 has the genotype MATa ura3Δ::loxP-kanMX-loxP.

HIS3 Deletion

The four fragments for the PCR cassette for the scarless HIS3 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen; Valencia, Calif.). HIS3 Fragment A was amplified with primer oBP452 (SEQ ID NO: 89) and primer oBP453 (SEQ ID NO:109), containing a 5' tail with homology to the 5' end of HIS3 Fragment B. HIS3 Fragment B was amplified with primer oBP454 (SEQ ID NO:110), containing a 5' tail with homology to the 3' end of HIS3 Fragment A, and primer oBP455 (SEQ ID NO:90), containing a 5' tail with homology to the 5' end of HIS3 Fragment U. HIS3 Fragment U was amplified with primer oBP456 (SEQ ID NO:91, containing a 5' tail with homology to the 3' end of HIS3 Fragment B, and primer oBP457 (SEQ ID NO:86), containing a 5' tail with homology to the 5' end of HIS3 Fragment C. HIS3 Fragment C was amplified with primer oBP458 (SEQ ID NO:87), containing a 5' tail with homology to the 3' end of HIS3 Fragment U, and primer oBP459 (SEQ ID NO:88). PCR products were purified with a PCR Purification kit (Qiagen). HIS3 Fragment AB was created by overlapping PCR by mixing HIS3 Fragment A and HIS3 Fragment B and amplifying with primers oBP452 (SEQ ID NO:89) and oBP455 (SEQ ID NO:90). HIS3 Fragment UC was created by overlapping PCR by mixing HIS3 Fragment U and HIS3 Fragment C and amplifying with primers oBP456 (SEQ ID NO:91) and oBP459 (SEQ ID NO:88). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The HIS3 ABUC cassette was created by overlapping PCR by mixing HIS3 Fragment AB and HIS3 Fragment UC and amplifying with primers oBP452 (SEQ ID NO:89) and oBP459 (SEQ ID NO:88). The final PCR product was purified with a PCR Purification kit (Qiagen).

To delete the endogenous HIS3 coding region, the "scarless" deletion cassette was transformed into NYLA106 using standard techniques and plated on synthetic complete media lacking uracil and supplemented with 2% glucose. Transformants were screened to verify correct integration by replica plating onto synthetic complete media lacking histidine and supplemented with 2% glucose at 30° C. Genomic DNA preps were made to verify the integration by PCR using primers BP460 and LA135 (SEQ ID NOs:93 and 94) for the 5' end and primers BP461 and LA92 (SEQ ID NOs:95 and 96) for the 3' end. The URA3 marker was recycled by plating on synthetic complete media supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA media to verify the absence of growth. The resulting identified strain, called PNY2003, has the genotype MATa ura3Δ::loxP-kanMX-loxP his3Δ.

Deletion of PDC1:

To delete the endogenous PDC1 coding region, a deletion cassette was PCR-amplified from pLA59 (SEQ ID NO:97), which contains a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using Phusion DNA polymerase and primers LA678 and LA679 (SEQ ID NOs:98 and 99). The PDC1 portion of each primer was derived from the 5' region 50 bp downstream of the PDC1 start codon and 3' region 50 bp upstream of the stop codon such that integration of the URA3 cassette results in replacement of the PDC1 coding region but leaves the first 50 bp and the last 50 bp of the coding region. The PCR product was transformed into strain PNY2003 using standard genetic techniques with selection on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. Transformants were screened to verify correct integration by colony PCR using primers LA337 (SEQ ID NO:111), external to the 5' coding region and LA135 (SEQ ID NO:94), an internal to URA3. Positive transformants were then screened by colony PCR using primers LA692 and LA693 (SEQ ID NOs:112 and 113), which were internal to the PDC1 coding region. The URA3 marker was recycled by transforming with pRS423::P_{GAL1}-cre (SEQ ID NO:121) and plated on synthetic complete media lacking histidine and supplemented with 2% glucose at 30° C. Transformants were plated on YP supplemented with 0.5% galactose to induce expression of Cre recombinase. Marker removal was

confirmed by patching colonies to synthetic complete media lacking uracil and supplemented with 2% glucose to verify absence of growth. The resulting identified strain, called PNY2008, has the genotype MATa ura3Δ::loxP-kanMX-loxP his3Δ pdc1Δ::loxP71/66.

Construction of ILV2-410 Integration Vector:

A fragment of the native ILV2 gene from *S. cerevisiae* BY4700 was PCR-amplified using Phusion DNA polymerase and primers LA684 and LA685 (SEQ ID NOs: 114 and 115). The ~2 kb PCR product was digested with BamHI and SphI and cloned into pUC19, and the resulting vector was named pUC19::ILV2 (SEQ ID No:17). Site-directed mutagenesis (QuickChange XL, Stratagene, CA) was used to introduce a C to T transition at base pair 574, resulting in a proline-to-serine substitution (Yadav et al. 1986 *PNAS.* 83:4418-4422). PfuUltra DNA polymerase (Stratagene, CA), primers LA682 and LA683 (SEQ ID NOs:116 and 117), and pUC19::ILV2 template were used to introduce the mutation following standard protocol. The PCR reaction was digested with DpnI to remove parental DNA, and the reaction was transformed into DH5α competent cells on LB-Amp (100 μg/ml). The presence of DNA containing the ILV2-410 allele was confirmed by DNA sequencing of plasmid DNA isolated from transformants. The resulting vector was named pUC19::ILV2-410.

The ILV2-410 fragment was digested from pUC19::ILV2-410 by BamHI SphI digest and subcloned into pLA59. pLA59 (SEQ ID No:97) is a pUC19 cloning vector that contains a loxP71-URA3-loxP66 cassette. The resulting vector, pLA59::ILV2-410 (SEQ ID NO:18), was used as template for PCR of the full integration cassette. The ILV2-410-loxP71-URA3-loxP66 integration cassette was PCR amplified from pLA59::ILV2-410 template using Phusion DNA polymerase and primers LA686 and LA687 (SEQ ID NOs:119 and 120), producing a ~3 kb product. The ILV2 portion of each primer was derived from the 5' region downstream of the ILV2 start codon and 3' region downstream of the stop codon such that integration of the URA3 cassette results in replacement of the native ILV2 coding region.

The PCR product was transformed into strain PNY2008 and plated on synthetic complete media lacking uracil and supplemented with 2% glucose at 30° C. The loxP71-URA3-loxP66 marker was recycled by transformation with pRS423::P_{GAL1}-cre (SEQ ID NO:121) and plating on synthetic complete media lacking histidine supplemented with 3% glucose at 30° C. Colonies were patched onto YP (1% galactose) plates at 30° C. to induce URA3 marker excision and were transferred onto YP (2% glucose) plates at 30° C. for recovery. Removal of the URA3 marker were confirmed by patching colonies from the YP (2% glucose) plates onto synthetic complete media lacking uracil supplemented with 2% glucose to verify the absence of growth. The resulting identified strain, called PNY2010, has the genotype MATa ura3Δ::loxP-kanMX-loxP his3Δ pdc1Δ::loxP71/66 ILV2-410::loxP71/66.

Example 4

Susceptibility of Wildtype *S. cerevisiae* Strains to Sulfonylurea Herbicides

This example describes experiments that demonstrate yeast strains, expressing wildtype acetolactate synthase, are resistant to many AHAS-inhibiting sulfonylurea herbicides. Strains tested in this experiment included: *S. cerevisiae* yeast strain PNY0860-1A), a haploid derived from sporulation of the yeast strain deposited with ATCC (ATCC #PTA-12007; *S. cerevisiae* yeast strain PNY827 (ATCC # PTA-12105); and *S. cerevisiae* strain CEN.PK 113-7D (Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre #8340).

The following AHAS inhibitors were resuspended in 10 mM KOH to give final concentrations of 2 mg/ml (w/v).

Accent ™	nicosulfuron methyl (V9360)
W4189-128	research sample
Ally ™	metsulfuron methyl (T6376)
Classic ™	chlorimuron ethyl (F6025)
Oust ™	sulfometuron methyl (SM)
Harmony ™	thifensulfuron methyl

The yeast strains were initially grown on synthetic complete liquid media lacking all amino acids and supplemented with 2% glucose at 30° C. Early stationary phase cultures (OD600 nm of ~5.0) were used to inoculate 40 ml of top agar media (synthetic complete lacking all amino acids with 0.7% agarose), which were poured into petri plates. Filter disks containing 20 μg AHAS inhibitor (20 p. 1 of a 1 mg/ml stock) or 10 μg AHAS inhibitor (20 μl of a 0.2 mg/ml stock) were added to the plate surface. Plates were incubated for 72 hours at 30° C. before visualization of zones of growth inhibition. Clear zones surrounding the AHAS-laced filter disks indicate inhibition of yeast growth. These results suggested that Classic, Ally, and (just herbicides inhibit growth of the yeast strains. Accent, Harmony, and W4189 did not inhibit the industrial yeast strains at the concentrations used in this experiment. (FIG. **2**)

Example 5

Resistance of Engineered *S. cerevisiae* Strains Containing an ILV2 Variant Gene to Sulfonylurea Herbicides

This example describes experiments that demonstrate yeast strains, expressing a resistant variant of acetolactate synthase, are resistant to the AHAS inhibitor sulfometuron methyl.

S. cerevisiae yeast strains PNY2008 and PNY2010 are described in Example 3. PNY2010 contains the ILV2-410 allele that confers resistance to sulfonylureas.

The yeast strains were initially grown on synthetic complete media supplemented with 2% glucose at 30° C. The strains were patched onto either synthetic complete media supplemented with 2% glucose at 30° C. or synthetic complete media supplemented with 2% glucose and 12.5 μg/ml sulfometuron methyl (prepared in 10 mM KOH as in Example 4). Plates were incubated for 48 hours at 30° C. before visualization. Strain PNY2008 was unable to grow on plates containing sulfometuron methyl, whereas strain PNY2010 grew normally due to the presence of the ILV2-410 allele.

Example 6

Production of Isobutanol in Recombinant *S. cerevisiae* NYLA84

The purpose of this example is to describe the production of isobutanol in the yeast strain NYLA84. The yeast strain comprises deletions of PDC1, PDC5, and PDC6, genes encoding three isozymes of pyruvate decarboxylase, and deletion of HXK2 encoding hexokinase II. The strain also

contains constructs for heterologous expression of AlsS (acetolactate synthase), KARI (keto acid reductoisomerase), DHAD (dihydroxy acid dehydratase), KivD (ketoisovalerate decarboxylase), and SadB (secondary alcohol dehydrogenase).

Strain Construction

Plasmids pLH468 and pLH475-JEA1 were introduced into NYLA84, described in Example 3, by standard PEG/lithium acetate-mediated transformation methods. Transformants were selected on synthetic complete medium lacking glucose, histidine and uracil. Ethanol (1% v/v) was used as the carbon source. After three days, transformants were patched to synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources. Freezer vials were made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

Production of Isobutanol

80 ml of synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources was inoculated with a yeast strain.

Fermentation Conditions:

Medium (final concentration): 6.7 g/L, Yeast Nitrogen Base w/o amino acids (Difco); 2.8 g/L, Yeast Synthetic Drop-out Medium Supplement Without Histidine, Leucine, Tryptophan and Uracil (Sigma Y2001); 20 mL/L of 1% (w/v) L-Leucine; 4 mL/L of 1% (w/v) L-Tryptophan; 1 mL/L ergosterol/tween/ethanol solution; 0.2 mL/L Sigma DF204; 10 g/L glucose

The fermenter was set to control at pH 5.5 with KOH, 30% dO, temperature 30° C., and airflow of 0.2 SLPM (or, 0.25 vvm). At inoculation, the airflow was set to 0.01 SLPM initially, then increased to 0.2 SLPM once growth was established. Glucose was maintained at 5-15 g/L throughout by manual addition.

Because efficient production of isobutanol with NYLA84 pLH486/pLH475 requires microaerobic conditions to enable redox balance in the biosynthetic pathway, air was continuously supplied to the fermenter at 0.25 vvm. Continuous aeration led to significant stripping of isobutanol from the aqueous phase of the fermenter. To quantify the loss of isobutanol due to stripping, the off-gas from the fermenter was directly sent to a mass spectrometer (Prima dB mass spectrometer, Thermo Electron Corp., Madison, Wis.) to quantify the amount of isobutanol in the gas stream. The isobutanol peaks at mass to charge ratios of 74 or 42 were monitored continuously to quantify the amount of isobutanol in the gas stream.

Glucose and organic acids in the aqueous phase were monitored during the fermentation using HPLC. Glucose was also monitored quickly using a glucose analyzer (YSI, Inc., Yellow Springs, Ohio). Isobutanol in the aqueous phase was quantified by HPLC as described in the General Methods Section herein above after the aqueous phase was removed periodically from the fermenter. The effective titer, corrected for the isobutanol lost due to stripping, was 7.5 g/L. The titer of isobutyric acid was 1.28 g/L. (FIG. **3**)

Example 7 (Prophetic):

Resistance of Engineered *S. cerevisiae* Isobutanologens Containing an IL V2 Variant Gene to Sulfonylurea Herbicides

This example describes experiments that demonstrate yeast strains that contain an engineered isobutanol production pathway which also express a resistant variant of acetolactate synthase, are resistant to the AHAS inhibitor sulfometuron methyl. Construction of strain NYLA84 is shown in Example 2.

The ILV2-410-loxP71-URA3-loxP66 integration cassette (described in Example 3) is PCR amplified from pLA59::ILV2-410 template using Phusion DNA polymerase and primers LA686 and LA687 (SEQ ID NOs:119 and 120), producing a ~3 kb product. The PCR product is transformed into strain NYLA84 and plated on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. The loxP71-URA3-loxP66 marker is recycled by transformation with pRS423::P_{GAL1}-cre (SEQ ID NO:121) and plating on synthetic complete media lacking histidine supplemented with 1% ethanol at 30° C. Colonies are patched onto YP (1% galactose) plates at 30° C. to induce URA3 marker excision and are transferred onto YP (1% ethanol) plates at 30° C. for recovery. Removal of the URA3 marker is confirmed by patching colonies from the YP (1% ethanol) plates onto synthetic complete media lacking uracil supplemented with 1% ethanol to verify the absence of growth. The resulting identified strain has the genotype NYLA84 ILV2-410::loxP71/66.

Plasmid vectors pLH468 and pLH475-JEA1 were simultaneously transformed into strain NYLA84 ILV2-410::loxP71/66 using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) and the resulting strain was maintained on synthetic complete media lacking histidine and uracil, and supplemented with 1% ethanol at 30° C.

The yeast strains are initially grown on synthetic complete media lacking histidine and uracil, and supplemented with 1% ethanol at 30° C. After three days, transformants are patched to synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources.

20 ml of synthetic complete medium lacking histidine and uracil supplemented with both 0.2% glucose and 0.5% ethanol as carbon sources at 30° C. is inoculated with the yeast strain. Each strain is diluted to an initial OD of 0.2 in tubes containing fresh synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources. The AHAS inhibitor sulfometuron methyl is added to the tubes at concentrations ranging from 0 μg/ml to 50 μg/ml. The tubes are incubated overnight at 30° C. shaking at 220 rpm and are scored the following day for growth. Strains expressing cytosolic acetolactate synthase demonstrate higher resistance to sulfometuron methyl. Isobutanol in the aqueous phase is quantified by HPLC as described in the General Methods Section.

Example 8 (Prophetic):

Production of Isobutanol in Recombinant *S. cerevisiae* NYLA84 in the Presence of Wildtype Yeast Competitor and Sulfometuron Methyl Herbicide

The purpose of this example is to describe the production of isobutanol in the yeast strain NYLA84 ILV2-410::loxP71/66 pLH468/pLH475-JEA1 when challenged with a wildtype yeast strain. The AHAS inhibitor sulfometuron methyl is added to prevent or retard growth of the wildtype yeast strain.

Strain Construction

Plasmids pLH468 and pLH475-JEA1 are introduced into NYLA84 ILV2-410::loxP71/66 pLH468/pLH475-JEA1, described in Example 7, by standard PEG/lithium acetate-

mediated transformation methods. Transformants are selected on synthetic complete medium lacking glucose, histidine and uracil. Ethanol (1% v/v) is used as the carbon source. After three days, transformants are patched to synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources.

Wildtype competitor strain Ethanol Red (Fermentis) is grown is synthetic complete medium supplemented with 2% glucose as carbon source.

Production of Isobutanol

80 ml of synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources is inoculated with the yeast strain.

Fermentation Conditions:

Medium (final concentration): 6.7 g/L, Yeast Nitrogen Base w/o amino acids (Difco); 2.8 g/L, Yeast Synthetic Drop-out Medium Supplement Without Histidine, Leucine Tryptophan and Uracil (Sigma Y2001); 20 mL/L of 1% (w/v) L-Leucine; 4 mL/L of 1% (w/v) L-Tryptophan; 1 mL/L ergosterol/tween/ethanol solution; 0.2 mL/L Sigma DF204; 10 g/L glucose.

Both fermenters are inoculated with NYLA84 pLH486/pLH475 and Ethanol Red (at 0.5× number of cells as the NYLA84 strain). Sulfometuron methyl is added to one fermenter at a concentration found to be inhibitory (see Example 4). The fermenters are set to control at pH 5.5 with KOH, 30% dO, temperature 30° C., and airflow of 0.2 SLPM (or, 0.25 vvm). At inoculation, the airflow is set to 0.01 SLPM initially, then increased to 0.2 SLPM once growth is established. Glucose is maintained at 5-15 g/L throughout by manual addition.

Because efficient production of isobutanol with NYLA84 pLH486/pLH475 requires microaerobic conditions to enable redox balance in the biosynthetic pathway, air is continuously supplied to the fermenter at 0.25 vvm. Continuous aeration leads to significant stripping of isobutanol from the aqueous phase of the fermenter. To quantify the loss of isobutanol due to stripping, the off-gas from the fermenter is directly sent to a mass spectrometer (Prima dB mass spectrometer, Thermo Electron Corp., Madison, Wis.) to quantify the amount of isobutanol in the gas stream. The isobutanol peaks at mass to charge ratios of 74 or 42 are monitored continuously to quantify the amount of isobutanol in the gas stream.

Glucose and organic acids in the aqueous phase are monitored during the fermentation using HPLC. Glucose is also monitored quickly using a glucose analyzer (YSI, Inc., Yellow Springs, Ohio). Isobutanol in the aqueous phase is quantified by HPLC as described in the General Methods Section herein above, after the aqueous phase is removed periodically from the fermenter.

Example 9 (Prophetic):

Isobutanol Production in an Engineered *S. cerevisiae* Isobutanologens Containing a Heterologous Acetolactate Synthase that is Resistant to Sulfonylurea Herbicides

This example describes experiments that demonstrate yeast strains, which contain an engineered isobutanol production pathway and express a heterologous acetolactate synthase that is resistant to sulfonylurea herbicides, are resistant to the AHAS inhibitor sulfometuron methyl. Construction of strain NYLA84 is shown in Example 2.

The enzyme ALS I (encoded by ilvB) from the enterobacteria *Escherichia coli* K12, which is intrinsically resistant to sulfonylurea herbicides, is PCR-amplified from *E. coli* K12 genomic DNA using Phusion DNA polymerase and primers T001 and T002 (SEQ ID NOs:122 and 123). The FBA1 promoter is PCR amplified from BY4700 genomic DNA using Phusion DNA polymerase and primers T003 and T004 (SEQ ID NOs:124 and 125). The FBA1 terminator is PCR amplified from BY4700 genomic DNA using Phusion DNA polymerase and primers T005 and T006 (SEQ ID NOs:126 and 127). The FBA1 promoter is digested with SphI KpnI, the ilvB gene is digested with KpnI NotI, and the FBA1 terminator is digested with NotI BamHI. The three fragments are ligated together and subcloned into vector pLA59 (described in Example 3) via SphI BamHI sites, creating vector pLA59::ilvB (SEQ ID NO:19).

The bdh1Δ::P_{FBA1}-ilvB-FBA1t-loxP71-URA3-loxP66 integration cassette is PCR amplified from pLA59::ilvB template (SEQ ID NO:19) using Phusion DNA polymerase and primers T007 and T008 (SEQ ID NOs:128 and 129). The BDH1 portion of each primer was derived from the 5' region 50 bp downstream of the BDH1 start codon and 3' region 50 bp upstream of the stop codon such that integration of the URA3 cassette results in replacement of the BDH1 coding region but leaves the first ~50 bp and the last ~50 bp of the coding region. The PCR product is transformed into strain NYLA84 and plated on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. The loxP71-URA3-loxP66 marker is recycled by transformation with pRS423::P_{GAL1}-cre (SEQ ID NO:121) and plating on synthetic complete media lacking histidine supplemented with 1% ethanol at 30° C. Colonies are patched onto YP (1% galactose) plates at 30° C. to induce URA3 marker excision and are transferred onto YP (1% ethanol) plates at 30° C. for recovery. Removal of the URA3 marker is confirmed by patching colonies from the YP (1% ethanol) plates onto synthetic complete media lacking uracil supplemented with 1% ethanol to verify the absence of growth. The resulting identified strain has the genotype NYLA84 bdh1::ilvB-loxP71/66.

Plasmid vectors pLH468 and pLH475-JEA1 are simultaneously transformed into strain NYLA84 bdh1::ilvB-loxP71/66 using standard genetic techniques (*Methods in Yeast Genetics,* 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) and the resulting strain is maintained on synthetic complete media lacking histidine and uracil, and supplemented with 1% ethanol at 30° C.

The yeast strains are initially grown on synthetic complete media lacking histidine and uracil, and supplemented with 1% ethanol at 30° C. After three days, transformants are patched to synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources.

20 ml of synthetic complete medium lacking histidine and uracil supplemented with both 0.2% glucose and 0.5% ethanol as carbon sources at 30° C. is inoculated with the yeast strain. Each strain is diluted to an initial OD of 0.2 in tubes containing fresh synthetic complete medium lacking histidine and uracil supplemented with both 2% glucose and 0.5% ethanol as carbon sources. The AHAS inhibitor sulfometuron methyl is added to the tubes at concentrations ranging from 0 μg/ml to 50 μg/ml. The tubes are incubated overnight at 30° C. shaking at 220 rpm and are scored the following day for growth. Strains expressing cytosolic acetolactate synthase demonstrate higher resistance to sulfometuron methyl. Isobutanol in the aqueous phase is quantified by HPLC as described in the General Methods Section.

Enterobacterial ALS enzymes are described in LaRossa and Smul, *J. Bacteriol.* 160(1):391-394 (1984). LaRossa describes ALSI enzymes from *S. typhimurium* and *E. coli* that are resistant to sulfonylurea herbicides.

Materials and Methods for Examples 10 to 20

Yeast synthetic medium w/o amino acids, w/o glucose, w/o ethanol/Tween (2×) 13.4 g/l, Yeast Nitrogen Base w/o amino acids (Difco 0919-15-3); 40 mg/L thiamine; 40 mg/L niacin; 200 ml/L 1M MES buffer, pH=5.5

Supplement aa sol. without histidine and uracil (SAAS-1 10×): 18.5 g/L, Synthetic complete amino acid dropout (Kaiser)-His, -Ura (Formedium).

Na-acetate stock solution: 3 M sodium acetate solution

Glucose stock solution: 250 g/L glucose solution

Inhibitor stock solutions: (1) copper (II) sulfate pentahydrate: CuSO4.5H2O (MW 249.6 g/mol, CAS Number 7758-99-8): 150 mM; (2) formaldehyde solution (SIGMA F8775, 36.5-38% in H2O, MW 30.03 g/mol, CAS Number 50-00-0): 12.15 M; (3) sodium sulfite (Na2SO3, SIGMA-ALDRICH 50505, CAS Number 7757-83-7, MW 126.04 g/mol): 100 mM in SF11, 500 mM in SF12; (4) bismuth(III) citrate (CAS Number 813-93-4, [O2CCH2C(OH)(CO2) CH2CO2] Bi, MW 398.08 g/mol): saturated solution; (5) sulfometuron methyl (Fluka #34224, CAS Number 74222-97-2, C15H16N4O5S, MW 364.38 g/mol): 10 g/L in DMSO; (6) 4-pyrazolecarboxylic acid (Sigma-Aldrich, #300713, C4H4N2O2, MW: 112.09 g/mol, CAS Number: 37718-11-9: 1.0 M in SF12 (=112 mg/ml (DMSO)); (7) 4-methylpyrazole hydrochloride (Sigma, # M1387, C4H6N2.HCl, MW: 118.56 g/mol, CAS: 56010-88-9): 1.0M in SF12 (=119 mg/ml (DMSO)); (8) pyrazole (Aldrich, # P56607, C3H4N2, MW: 68.08 g/mol, CAS Number: 288-13-1): 0.5 M in SF12 (=34 mg/ml); (9) glyoxylic acid sodium salt monohydrate (HC(O)COONa.H2O, MW: 114.03, CAS Number: 918149-31-2): 0.5 M in SF12 (=57 mg/ml); (10) pyrazole (Aldrich, # P56607, C3H4N2, MW: 68.08 g/mol, CAS Number: 288-13-1): 0.5 M (=34 mg/ml) in SF13; (11) trans-cinnamaldehyde (Aldrich #239968, C6H5CH═CHCHO, MW: 132.16 g/mol, CAS: 14371-10-9, d=1.050 g/ml): SF12 and SF13=pure liquid, SF14=20 mM in DMSO; (12) 1-bromo-2-butanone (Sigma-Aldrich #243299, C2H5COCH2Br, MW: 151.00 g/mol, CAS: 816-40-0, d=1.479 g/l): SF12 and SF13=pure liquid, SF14=10 mM in DMSO; (13) 4-nitrocinnamaldehyde (predominantly "trans" form, Aldrich #281670, O2NC6H4CH═CHCHO, MW: 177.16 g/mol, CAS: 49678-08-2): SF12=pure substance was weighted and added to the culture

SEED medium: 10,000 mL Yeast synthetic medium w/o aa, w/o glucose, w/o ethanol/Tween (2×); 2.000 mL Supplement aa sol. without histidine and uracil (SAAS-1 10×); 3.200 mL 250 g/L glucose solution (resulting in 40 g/l glucose); 0.046 mL Na-acetate stock solution; 4.754 mL H2O.

Example 10

Inhibition of Ethanologen Yeast PNY 827 by Copper(II)

The inhibitory effect of copper (II) on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without copper or addition of copper at concentrations of 5 mM, 10 mM and 25 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3). The solution with 25 mM showed precipitation and was not analyzed. At 5 mM of copper μ_{max} was determined to be 0.46 l/h. In the medium containing 10 mM of copper, maximum specific growth rate of μ_{max}=0.33 l/h was found. Fitting the data to the "squared inhibition" kinetics yielded parameters of $\mu°_{max}$=0.58 l/h and a K_I value of K_I=11 mM (FIG. **4**). Decrease in μ_{max} with increasing copper (II) concentrations in the medium indicates inhibition of ethanologen yeast PNY 827 Inhibition kinetics were used and fitted parameters predict an IC_{50} value of 11 mM. Data from the samples is seen in Table 3 below.

TABLE 3

Data for control samples and copper-inhibited experiments.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-ctrl-a							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.157	1	0.090	
2	10:40	135	2.25	0.188	1	0.121	0.121
3	11:40	195	3.25	0.255	1	0.188	0.188
4	12:40	255	4.25	0.372	1	0.305	0.305
5	14:25	360	6.00	0.285	5	1.102	1.102
6	8:05	1420	23.67	0.672	20	12.157	
F2-ctrl-b							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.153	1	0.086	

TABLE 3-continued

Data for control samples and copper-inhibited experiments.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
2	10:40	135	2.25	0.187	1	0.120	0.120
3	11:40	195	3.25	0.251	1	0.184	0.184
4	12:40	255	4.25	0.371	1	0.304	0.304
5	14:25	360	6.00	0.281	5	1.082	1.082
6	8:05	1420	23.67	0.647	20	11.657	
				F5-cu-1			
0	8:25	0	0.00	0.336	1	0.097	
1	9:25	60	1.00	0.346	1	0.107	0.107
2	10:40	135	2.25	0.467	1	0.228	0.228
3	11:40	195	3.25	0.538	1	0.299	0.299
4	12:40	255	4.25	0.543	1	0.304	
5	14:25	360	6.00	0.152	5	0.265	
6	18:05	580	9.67	0.163	5	0.320	
6	8:05	1420	23.67	0.171	5	0.360	
				F6-cu-2			
0	8:25	0	0.00	0.389	1	0.115	
1	9:25	60	1.00	0.399	1	0.125	
2	10:40	135	2.25	0.401	1	0.127	0.127
3	11:40	195	3.25	0.437	1	0.163	0.163
4	12:40	255	4.25	0.521	1	0.247	0.247
5	14:25	360	6.00	0.158	5	0.260	
6	18:05	580	9.67	0.166	5	0.300	
6	8:05	1420	23.67	0.202	5	0.480	
				F7-cu-3			
precipitation							

Copper concentrations in the experiments were:
F1-ctrl-a: 0 mM;
F2-ctrl-b: 0 mM;
F5-cu-1: 5 mM;
F6-cu-2: 10 mM;
F7-cu-3: 25 mM.

Example 11

Inhibition of Ethanologen Yeast PNY 827 by Sulfometuron Methyl

The inhibitory effect of the sulfonylurea sulfometuron methyl on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without sulfometuron methyl or addition of sulfometuron methyl at concentrations of 0.11 mM, 0.16 mM and 0.27, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

At all three applied concentrations of 0.11 mM, 0.16 mM and 0.27 mM of sulfometuron methyl a significant reduction in specific maximum growth rate was found, yielding μ_{max} values of 0.04 l/h, 0.05 l/h and 0.04 l/h, down from uninhibited maximum specific growth in the experiment of μ°_{max}=0.59 l/h, respectively (5).

Sulfometuron methyl is poorly water soluble, consequently the compound was administered dissolved in DMSO. In order to make sure the observed inhibition did not result from DMSO, DMSO was added only ad 0.14 mM to a culture and a maximum specific growth rate of 0.56 l/h was found. Follow up experiments with DMSO point to a “squared inhibition” with a K_I value of about 16 mM (data not shown). So while DMSO alone seems to have an inhibitory effect on growth, its inhibitory effects at concentrations of 0.06 mM, 0.08 mM and 0.14 mM, as used in the experiments with sulfometuron methyl, can be neglected. Fitting a “hybrid” inhibition kinetics model to the measurements yields values of $\mu^{\circ}_{max\ 1}$=0.55 l/h and $\mu^{\circ}_{max\ 2}$=0.04 l/h. Not sufficient data are available for accurate determination of K_I, but from the curve shape it can be concluded that the K_I value is significantly below 0.1 mM. The “hybrid” inhibition kinetics model predicts an overall observable μ°_{max} of 0.59 l/h. Due to the underdetermined K_I value, the IC_{50} value cannot reliably be determined, but it can be concluded that it is significantly lower than 0.1 mM. Data from the samples is seen in Table 4 below.

TABLE 4

Data for control samples and sulfometuron methyl-inhibited experiments. Sulfometuron methyl concentrations in the experiments were: F1-ctrl-a: 0 mM; F2-ctrl-b: 0 mM; F12-sm-1: 0.11 mM; F13-sm-2: 0.16 mM; F14-sm-3: 0.27 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-ctrl-a							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.157	1	0.090	
2	10:40	135	2.25	0.188	1	0.121	0.121
3	11:40	195	3.25	0.255	1	0.188	0.188
4	12:40	255	4.25	0.372	1	0.305	0.305
5	14:25	360	6.00	0.285	5	1.102	1.102
6	8:05	1420	23.67	0.672	20	12.157	
F2-ctrl-b							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.153	1	0.086	
2	10:40	135	2.25	0.187	1	0.120	0.120
3	11:40	195	3.25	0.251	1	0.184	0.184
4	12:40	255	4.25	0.371	1	0.304	0.304
5	14:25	360	6.00	0.281	5	1.082	1.082
6	8:05	1420	23.67	0.647	20	11.657	
F12-sm-1							
0	8:25	0	0.00	0.155	1	0.088	
1	9:25	60	1.00	0.160	1	0.093	0.093
2	10:40	135	2.25	0.165	1	0.098	0.098
3	11:40	195	3.25	0.170	1	0.103	0.103
4	12:40	255	4.25	0.175	1	0.108	0.108
5	14:25	360	6.00	0.182	1	0.115	0.115
6	17:50	565	9.42	0.222	1	0.155	
7	8:05	1420	23.67	0.272	5	1.037	
F13-sm-2							
0	8:25	0	0.00	0.158	1	0.091	
1	9:25	60	1.00	0.160	1	0.093	0.093
2	10:40	135	2.25	0.166	1	0.099	0.099
3	11:40	195	3.25	0.171	1	0.104	0.104
4	12:40	255	4.25	0.175	1	0.108	0.108
5	14:25	360	6.00	0.184	1	0.117	0.117
6	17:50	565	9.42	0.219	1	0.152	
7	8:05	1420	23.67	0.467	1	0.400	
F14-sm-3							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.157	1	0.090	0.090
2	10:40	135	2.25	0.164	1	0.097	0.097
3	11:40	195	3.25	0.164	1	0.097	0.097
4	12:40	255	4.25	0.166	1	0.099	0.099
5	14:25	360	6.00	0.177	1	0.110	0.110
6	17:50	565	9.42	0.201	1	0.134	0.134
7	8:05	1420	23.67	0.306	1	0.239	0.239

Example 12

Inhibition of Ethanologen Yeast PNY 827 by Sulfite

The inhibitory effect of sulfite on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without sulfite or addition of sulfite at concentrations of 1 mM, 2 mM, 5 mM, 10 mM, 20 mM and 50 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

Sulfite concentrations of 1 mM, 2 mM, 5 mM, 10 mM, 20 mM and 50 mM resulted in maximum specific growth rates of 0.59 l/h, 0.54 l/h, 0.33 l/h, 0.23 l/h, 0.18 l/h and 0.14 l/h, respectively, indicating significant inhibitory effect of sulfite on ethanologen yeast PNY 807. Fitting the measured data to the "hybrid" inhibition kinetics model, values of $\mu^{o}_{max\ 1}$=0.46 l/h, $\mu^{o}_{max\ 2}$=0.15 l/h and KI=4.4 mM were determined. The "hybrid" inhibition kinetics model predicts an overall observable μ^{o}_{max}=0.61 l/h and an IC50 value of 6.2 mM. Measured μ_{max} values and fitted dependency of μ_{max} on the concentration of sulfite in the medium is depicted in FIG. **6**. Data from the samples is seen in Table 5 below.

TABLE 5

Data for control samples and sulfite-inhibited experiments. Sulfite concentrations in the experiments were: F1-ctrl-a: 0 mM; F2-ctrl-b: 0 mM; F8-su-1: 1 mM; F9-su-2: 2 mM; F10-su-3: 5 mM; F11-su-4: 10 mM; SF12-F1-CtrlA: 0 mM; SF12-F2-ctrlB: 0M; F3-su-1: 20 mM; F4-su-2: 50 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-ctrl-a							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.157	1	0.090	
2	10:40	135	2.25	0.188	1	0.121	0.121
3	11:40	195	3.25	0.255	1	0.188	0.188
4	12:40	255	4.25	0.372	1	0.305	0.305
5	14:25	360	6.00	0.285	5	1.102	1.102
6	8:05	1420	23.67	0.672	20	12.157	
F2-ctrl-b							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.153	1	0.086	
2	10:40	135	2.25	0.187	1	0.120	0.120
3	11:40	195	3.25	0.251	1	0.184	0.184
4	12:40	255	4.25	0.371	1	0.304	0.304
5	14:25	360	6.00	0.281	5	1.082	1.082
6	8:05	1420	23.67	0.647	20	11.657	
F8-su-1							
0	8:25	0	0.00	0.148	1	0.081	
1	9:25	60	1.00	0.149	1	0.082	
2	10:40	135	2.25	0.188	1	0.121	0.121
3	11:40	195	3.25	0.252	1	0.185	0.185
4	12:40	255	4.25	0.374	1	0.307	0.307
5	14:25	360	6.00	0.285	5	1.102	1.102
6	8:05	1420	23.67	0.622	20	11.157	
F9-su-2							
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.153	1	0.086	
2	10:40	135	2.25	0.187	1	0.120	0.120

TABLE 5-continued

Data for control samples and sulfite-inhibited experiments. Sulfite concentrations in the experiments were: F1-ctrl-a: 0 mM; F2-ctrl-b: 0 mM; F8-su-1: 1 mM; F9-su-2: 2 mM; F10-su-3: 5 mM; F11-su-4: 10 mM; SF12-F1-CtrlA: 0 mM; SF12-F2-ctrlB: 0M; F3-su-1: 20 mM; F4-su-2: 50 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
3	11:40	195	3.25	0.240	1	0.173	0.173
4	12:40	255	4.25	0.332	1	0.265	0.265
5	14:25	360	6.00	0.242	5	0.887	0.887
6	8:05	1420	23.67	0.675	20	12.217	
				F10-su-3			
0	8:25	0	0.00	0.155	1	0.088	
1	9:25	60	1.00	0.157	1	0.090	
2	10:40	135	2.25	0.183	1	0.116	0.116
3	11:40	195	3.25	0.220	1	0.153	0.153
4	12:40	255	4.25	0.275	1	0.208	0.208
5	14:25	360	6.00	0.459	1	0.392	0.392
6	18:00	575	9.58	0.579	5	2.572	
7	8:05	1420	23.67	0.650	20	11.717	
				F11-su-4			
0	8:25	0	0.00	0.159	1	0.092	
1	9:25	60	1.00	0.152	1	0.085	0.085
2	10:40	135	2.25	0.178	1	0.111	0.111
3	11:40	195	3.25	0.207	1	0.140	0.140
4	12:40	255	4.25	0.242	1	0.175	0.175
5	14:25	360	6.00	0.335	1	0.268	0.268
6	18:00	575	9.58	0.285	5	1.102	
6	8:05	1420	23.67	0.636	20	11.437	
				SF12-F1-Ctrl-A			
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
				SF12-F2-ctrl-B			
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
				F3-su-1			
0	8:55	0	0.00	0.154	1	0.087	
1	10:25	90	1.50	0.166	1	0.099	
2	11:35	160	2.67	0.182	1	0.115	0.115
3	12:55	240	4.00	0.217	1	0.150	0.150
4	14:15	320	5.33	0.249	1	0.182	0.182
5	15:40	405	6.75	0.293	1	0.226	0.226
6	18:20	565	9.42	0.463	1	0.396	0.396
7	8:30	1395	23.25	0.549	20	9.697	
				F4-su-2			
0	8:55	0	0.00	0.155	1	0.088	
1	10:25	90	1.50	0.162	1	0.095	
2	11:35	160	2.67	0.183	1	0.116	0.116
3	12:55	240	4.00	0.202	1	0.135	0.135
4	14:15	320	5.33	0.228	1	0.161	0.161
5	15:40	405	6.75	0.259	1	0.192	0.192
6	18:20	565	9.42	0.362	1	0.295	0.295
7	8:30	1395	23.25	0.600	20	10.717	

Example 13

Inhibition of Ethanologen Yeast PNY 827 by Formaldehyde

The inhibitory effect of formaldehyde on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without formaldehyde or addition of formaldehyde at concentrations of 1 mM, 2 mM, 5 mM and 10 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

With the investigated formaldehyde concentrations of 1 mM, 2 mM, 5 mM and 10 mM, corresponding maximum specific growth rates of PNY827 were 0.28 l/h, 0.08 l/h, 0.00 l/h (no growth), and 0.00 l/h (no growth), respectively. Mumax values determined without inhibitor addition were 0.59 l/h and 0.59 l/h. Fitting the measured data to the "squared inhibition" kinetics model, a K_I value of K_I=904 μM (μ^{o}_{max}=0.59 l/h) was found, indicating a very strong inhibition of *S. cerevisiae* by formaldehyde. The derived ICSO value is 0.9 mM. Measured μ_{max} values and fitted dependency of μ_{max} on the concentration of formaldehyde in the medium is depicted in FIG. **7**. Data from the samples is seen in Table 6 below.

TABLE 6

Data for control samples and formaldehyde-inhibited experiments. Formaldehyde concentrations in the experiments were: F1-ctrl-a: 0 mM; F2-ctrl-b: 0 mM; F16-fa-1: 1 mM; F17-fa-2: 2 mM; F18-fa-3: 5 mM; F19-fa-4: 10 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
				F1-ctrl-a			
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.157	1	0.090	
2	10:40	135	2.25	0.188	1	0.121	0.121
3	11:40	195	3.25	0.255	1	0.188	0.188
4	12:40	255	4.25	0.372	1	0.305	0.305
5	14:25	360	6.00	0.285	5	1.102	1.102
6	8:05	1420	23.67	0.672	20	12.157	
				F2-ctrl-b			
0	8:25	0	0.00	0.154	1	0.087	
1	9:25	60	1.00	0.153	1	0.086	
2	10:40	135	2.25	0.187	1	0.120	0.120
3	11:40	195	3.25	0.251	1	0.184	0.184
4	12:40	255	4.25	0.371	1	0.304	0.304
5	14:25	360	6.00	0.281	5	1.082	1.082
6	8:05	1420	23.67	0.647	20	11.657	

TABLE 6-continued

Data for control samples and formaldehyde-inhibited experiments. Formaldehyde concentrations in the experiments were: F1-ctrl-a: 0 mM; F2-ctrl-b: 0 mM; F16-fa-1: 1 mM; F17-fa-2: 2 mM; F18-fa-3: 5 mM; F19-fa-4: 10 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F16-fa-1							
0	8:35	0	0.00	0.157	1	0.090	
1	9:35	60	1.00	0.157	1	0.090	
2	10:50	135	2.25	0.165	1	0.098	
3	11:50	195	3.25	0.182	1	0.115	0.115
4	12:50	255	4.25	0.206	1	0.139	0.139
5	14:35	360	6.00	0.285	1	0.218	0.218
6	17:40	545	9.08	0.655	1	0.588	0.588
7	8:20	1425	23.75	0.657	20	11.857	
F17-fa-2							
0	8:35	0	0.00	0.158	1	0.091	
1	9:35	60	1.00	0.158	1	0.091	
2	10:50	135	2.25	0.159	1	0.092	
3	11:50	195	3.25	0.160	1	0.093	0.093
4	12:50	255	4.25	0.165	1	0.098	0.098
5	14:35	360	6.00	0.177	1	0.110	0.110
6	17:40	545	9.08	0.211	1	0.144	0.144
7	8:20	1425	23.75	0.382	20	6.357	
F18-fa-3							
0	8:35	0	0.00	0.157	1	0.090	
1	9:35	60	1.00	0.162	1	0.095	
2	10:50	135	2.25	0.161	1	0.094	
3	11:50	195	3.25	0.157	1	0.090	0.090
4	12:50	255	4.25	0.155	1	0.088	0.088
5	14:35	360	6.00	0.155	1	0.088	0.088
6	17:40	545	9.08	0.156	1	0.089	0.089
7	8:20	1425	23.75	0.160	1	0.093	
F19-fa-4							
0	8:35	0	0.00	0.162	1	0.095	
1	9:35	60	1.00	0.168	1	0.101	
2	10:50	135	2.25	0.164	1	0.097	
3	11:50	195	3.25	0.163	1	0.096	0.096
4	12:50	255	4.25	0.160	1	0.093	0.093
5	14:35	360	6.00	0.161	1	0.094	0.094
6	17:40	545	9.08	0.162	1	0.095	0.095
7	8:20	1425	23.75	0.168	1	0.101	

Example 14

Inhibition of Ethanologen Yeast PNY 827 by 4-pyrazolecarboxylic acid

The inhibitory effect of 4-nitrocinnamaldehyde (predominantly trans) on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without 4-pyrazolecarboxylic acid or addition of 4-nitrocinnamaldehyde at concentrations of 1 mM and 50 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

The inhibitory effect of 4-pyrazolecarboxylic acid (PA) was investigated at 1 mM and 50 mM. 4-pyrazolecarboxylic acid was administered as a DMSO solution, resulting in DMSO concentrations in the cell suspension of 14 mM and 704 mM, respectively. Assuming an additive effect of 4-pyrazolecarboxylic acid and DMSO inhibition, observed maximum specific growth rate of the two cultures was corrected by 0.00 l/h and 0.27 l/h due to the effect of DMSO, resulting in 4-pyrazolecarboxylic acid-based mumax values of 0.59 l/h and 0.50 l/h derived from the observed values of 0.59 l/h and 0.23 l/h, respectively. Fitting the data to the "squared inhibition" kinetics (observed maximum specific growth rates without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h and 0.62 l/h) yielded parameters of $\mu°_{max}$=0.60 l/h and a KI value of KI=100 mM (FIG. **8**), indicating only weak inhibitory effects of 4-pyrazolecarboxylic acid. Data from the samples is seen in Table 7 below.

TABLE 7

Data for control samples and 4-pyrazolecarboxylic acid - inhibited experiments. 4-pyrazolecarboxylic acid concentrations in the experiments were: F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F12-pa-1: 1 mM; F13-pa-2: 50 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-Ctrl-A							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
F2-ctrl-B							
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
F12-pa-1							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.227	1	0.160	0.160
3	12:55	240	4.00	0.361	1	0.294	0.294
4	14:15	320	5.33	0.217	5	0.762	0.762
5	8:30	1395	23.25	0.659	20	11.897	
F13-pa-2							
0	8:55	0	0.00	0.157	1	0.090	
1	10:25	90	1.50	0.167	1	0.100	0.100
2	11:35	160	2.67	0.192	1	0.125	0.125
3	12:55	240	4.00	0.234	1	0.167	0.167
4	14:15	320	5.33	0.305	1	0.238	0.238
5	3:40	405	6.75	0.405	1	0.338	0.338
6	8:30	1395	23.25	0.655	20	11.817	

Example 15

Inhibition of Ethanologen Yeast PNY 827 by 4-methylpyrazole hydrochloride

The inhibitory effect of 4-methylpyrazole hydrochloride on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without 4-methylpyrazole hydrochloride or addition of 4-methylpyrazole hydrochloride at concentrations of 1 mM and 30 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

The inhibitory effect of 4-methylpyrazole hydrochloride was investigated at 1 mM and 30 mM. 4-methylpyrazole hydrochloride was administered as a DMSO solution, resulting in DMSO concentrations in the cell suspension of 14 mM and 423 mM, respectively. Assuming an additive effect of 4-methylpyrazole hydrochloride and DMSO inhibition, observed maximum specific growth rate of the two cultures was corrected by 0.00 l/h and 0.14 l/h due to the effect of DMSO, resulting in 4-methylpyrazole hydrochloride-based mumax values of 0.48 l/h and 0.38 l/h derived from the observed values of 0.48 l/h and 0.24 l/h, respectively. Fitting the data to the "squared inhibition" kinetics (observed maximum specific growth rates without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h and 0.62 l/h) yielded parameters of μ°_{max}=0.58 l/h and a K_I value of K_I=41 mM, indicating inhibitory effects of 4-methylpyrazole hydrochloride (9). Data from the samples is seen in Table 8 below.

TABLE 8

Data for control samples and 4-methylpyrazole hydrochloride acid-inhibited experiments. 4-methylpyrazole hydrochloride acid concentrations in the experiments were: F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F14-mp-1: 1 mM; F15-mp-2: 30 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-Ctrl-A							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
F2-ctrl-B							
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
F14-mp-1							
0	8:55	0	0.00	0.163	1	0.096	
1	10:25	90	1.50	0.174	1	0.107	0.107
2	11:35	160	2.67	0.221	1	0.154	0.154
3	12:55	240	4.00	0.342	1	0.275	0.275
4	14:15	320	5.33	0.203	5	0.692	0.692
5	8:30	1395	23.25	0.626	20	11.237	
F15-mp-2							
0	8:55	0	0.00	0.161	1	0.094	
1	10:25	90	1.50	0.172	1	0.105	0.105
2	11:35	160	2.67	0.189	1	0.122	0.122
3	12:55	240	4.00	0.237	1	0.170	0.170
4	14:15	320	5.33	0.305	1	0.238	0.238
5	3:40	405	6.75	0.434	1	0.367	0.367
6	8:30	1395	23.25	0.729	20	13.297	

Example 16

Inhibition of Ethanologen Yeast PNY 827 by Glyoxylic Acid

The inhibitory effect of glyoxylic acid on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without glyoxylic acid or addition of glyoxylic acid at concentrations of 10 mM and 50 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from

a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

The inhibitory effect of glyoxylic acid was investigated at 10 mM and 50 mM. At the two concentrations, mumax values of 0.59 l/h and 0.55 l/h were found, respectively. Fitting the data to the "squared inhibition" kinetics (observed maximum specific growth rates without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h and 0.62 l/h) yielded parameters of $\mu°_{max}$=0.60 l/h and a K_I value of K_I=168 mM, indicating a weak inhibitory effect of extracellular glyoxylic acid on growth of ethanologen yeast (FIG. **10**.). Data from the samples is seen in Table 9 below.

TABLE 9

Data for control samples and glyoxylic acid-inhibited experiments. Glyoxylic acid concentrations in the experiments were: F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F18-ga-1: 10 mM; F19-ga-2: 50 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F1-Ctrl-A							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
F2-ctrl-B							
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
F18-ga-1							
0	8:55	0	0.00	0.161	1	0.094	
1	10:25	90	1.50	0.175	1	0.108	
2	11:35	160	2.67	0.226	1	0.159	0.159
3	12:55	240	4.00	0.361	1	0.294	0.294
4	14:15	320	5.33	0.217	5	0.762	0.762
5	8:30	1395	23.25	0.634	20	11.397	
F19-ga-2							
0	8:55	0	0.00	0.157	1	0.090	
1	10:25	90	1.50	0.173	1	0.106	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.337	1	0.270	0.270
4	14:15	320	5.33	0.199	5	0.672	0.672
5	8:30	1395	23.25	0.655	20	11.817	

Example 17

Inhibition of Ethanologen Yeast PNY 827 by Pyrazole

The inhibitory effect of pyrazole on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without pyrazole or addition of pyrazole at concentrations of 1 mM, 5 mM, 10 mM, 25 mM and 50 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

Pyrazole concentrations of 1 mM, 5 mM, 10 mM, 25 mM and 50 mM were tested, resulting in maximum specific growth rates of 0.54 l/h, 0.21 l/h, 0.12 l/h, 0.09 l/h and 0.08 l/h. Mumax values determined without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h, 0.62 l/h, 0.61 l/h and 0.62 l/h, respectively. Inhibitory effect of pyrazole on growth was best described by the hybrid growth model. If fitted to the "hybrid" inhibition kinetics model, values of $\mu°_{max\ 1}$=0.52 l/h, $\mu°_{max\ 2}$=0.08 l/h and K_I=2.8 mM were determined. The "hybrid" inhibition kinetics model predicts an overall observable $\mu°_{max}$=0.60 l/h and an IC_{50} (inhibitor concentration with a specific growth rate of 50% $\mu°_{max}$) value of 3.3 mM. Measured μ_{max} values and fitted dependency of μ_{max} on the concentration of pyrazole in the medium is depicted in FIG. **11**. Data from the samples is seen in Table 10 below.

TABLE 10

Data for control samples and pyrazole-inhibited experiments. Pyrazole concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F16-py-1: 1 mM; F17-py-2: 50 mM; F1-ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F8-Py-5: 5 mM; F9-Py-10: 10 mM; F10-Py-25: 25 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
SF12-F1-Ctrl-A							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
SF12-F2-ctrl-B							
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
F16-py-1							
0	8:55	0	0.00	0.155	1	0.088	
1	10:25	90	1.50	0.167	1	0.100	
2	11:35	160	2.67	0.199	1	0.132	0.132
3	12:55	240	4.00	0.277	1	0.210	0.210
4	14:15	320	5.33	0.444	1	0.377	0.377
5	3:40	405	6.75	0.262	5	0.987	0.987
6	8:30	1395	23.25	0.645	20	11.617	

TABLE 10-continued

Data for control samples and pyrazole-inhibited experiments. Pyrazole concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F16-py-1: 1 mM; F17-py-2: 50 mM; F1-ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F8-Py-5: 5 mM; F9-Py-10: 10 mM; F10-Py-25: 25 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
F17-py-2							
0	8:55	0	0.00	0.164	1	0.097	
1	10:25	90	1.50	0.166	1	0.099	0.099
2	11:35	160	2.67	0.169	1	0.102	0.102
3	12:55	240	4.00	0.175	1	0.108	0.108
4	14:15	320	5.33	0.186	1	0.119	0.119
5	15:40	405	6.75	0.190	1	0.123	0.123
6	18:20	565	9.42	0.209	1	0.142	0.142
7	8:30	1395	23.25	0.302	1	0.235	0.235
0	8:55	0	0.00	0.164	1	0.097	
1	10:25	90	1.50	0.166	1	0.099	0.099
2	11:35	160	2.67	0.169	1	0.102	0.102
3	12:55	240	4.00	0.175	1	0.108	0.108
4	14:15	320	5.33	0.186	1	0.119	0.119
5	15:40	405	6.75	0.190	1	0.123	0.123
6	18:20	565	9.42	0.209	1	0.142	0.142
7	8:30	1395	23.25	0.302	1	0.235	0.235
0	8:55	0	0.00	0.164	1	0.097	
1	10:25	90	1.50	0.166	1	0.099	0.099
2	11:35	160	2.67	0.169	1	0.102	0.102
3	12:55	240	4.00	0.175	1	0.108	0.108
4	14:15	320	5.33	0.186	1	0.119	0.119
5	15:40	405	6.75	0.190	1	0.123	0.123
6	18:20	565	9.42	0.209	1	0.142	0.142
7	8:30	1395	23.25	0.302	1	0.235	0.235
F1-ctrl-A							
0	8:15	0	0.00	0.160	1	0.093	
1	9:45	90	1.50	0.169	1	0.102	
2	11:05	170	2.83	0.229	1	0.162	0.162
3	12:20	245	4.08	0.368	1	0.301	0.301
4	13:35	320	5.33	0.215	5	0.752	0.752
6	8:30	1455	24.25	0.644	20	11.597	
F2-ctrl-B							
0	8:15	0	0.00	0.159	1	0.092	
1	9:45	90	1.50	0.168	1	0.101	
2	11:05	170	2.83	0.228	1	0.161	0.161
3	12:20	245	4.08	0.372	1	0.305	0.305
4	13:35	320	5.33	0.215	5	0.752	0.752
5	8:30	1455	24.25	0.652	20	11.757	
F8-Py-5							
0	8:15	0	0.00	0.163	1	0.096	
1	9:45	90	1.50	0.162	1	0.095	0.095
2	11:05	170	2.83	0.177	1	0.110	0.110
3	12:20	245	4.08	0.210	1	0.143	0.143
4	13:35	320	5.33	0.256	1	0.189	0.189
5	3:00	405	6.75	0.362	1	0.295	0.295
6	8:30	1455	24.25	0.551	20	9.737	
F9-Py-10							
0	8:15	0	0.00	0.164	1	0.097	
1	9:45	90	1.50	0.166	1	0.099	0.099
2	11:05	170	2.83	0.181	1	0.114	0.114
3	12:20	245	4.08	0.205	1	0.138	0.138
4	13:35	320	5.33	0.238	1	0.171	0.171
5	3:00	405	6.75	0.289	1	0.222	0.222
	8:30	1455	24.25	0.475	20	8.217	
F10-Py-25							
0	8:15	0	0.00	0.162	1	0.095	
1	9:45	90	1.50	0.159	1	0.092	0.092
2	11:05	170	2.83	0.167	1	0.100	0.100
3	12:20	245	4.08	0.181	1	0.114	0.114
4	13:35	320	5.33	0.194	1	0.127	0.127
5	3:00	405	6.75	0.211	1	0.144	0.144
	8:30	1455	24.25	0.374	20	6.197	

Example 18

Inhibition of Ethanologen Yeast PNY 827 by Cinnamaldehyde

The inhibitory effect of cinnamaldehyde on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated over night at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without cinnamaldehyde or addition of cinnamaldehyde at concentrations of 200 mM, 100 mM, 50 mM, 25 mM, 10 mM, 1 mM, 0.1 mM, 0.01 mM and 0.001 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

The inhibitory effect of cinnamaldehyde (CA) was investigated at 200 mM, 100 mM, 50 mM, 25 mM, 10 mM, 1 mM, 0.1 mM, 0.01 mM and 0.001 mM. For generating the concentrations of 0.1 mM, 0.01 mM and 0.001 mM, cinnamaldehyde was diluted with DMSO, resulting in DMSO concentrations in the cell suspension of 0.7 mM, 7 mM and 70 mM of DMSO, respectively. Assuming an additive effect of cinnamaldehyde and DMSO inhibition, observed maximum specific growth rates of the two cultures were corrected by 0.000 l/h, 0.000 l/h and 0.005 l/h due to the effect of DMSO, resulting in cinnamaldehyde-based mumax values of 0.64 l/h, 0.63 l/h and 0.55 l/h derived from the observed values of 0.64 l/h, 0.63 l/h and 0.55 l/h, respectively. At all the other concentrations, no DMSO was used in the stock solution of cinnamaldehyde. However, in none of the non-DMSO experiments any cell growth was observed. Fitting the data to the "squared inhibition" kinetics (observed maximum specific growth rates without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h, 0.62 l/h, 0.62 l/h and 0.62 l/h) yielded parameters of μ°_{max}=0.62 l/h and a K_I value of K_I=0.25 mM (FIG. **12**). These findings indicate strong inhibition of growth of PNY 827 by trans-cinnamaldehyde with a derived IC50 of 0.25 mM. Data from the samples is seen in Table 11 below.

TABLE 11

Data for control samples and cinnamaldehyde-inhibited experiments. Cinnamaldehyde concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F7-ca-1: 50 mM; F8-ca-2: 100 mM; F9-ca-3: 200 mM; SF13-F1-ctrl-A: 0 mM; SF13-F2-ctrl-B: 0 mM; F3-Ca-1: 1 mM; F4-Ca-10: 10 mM; F5-Ca-25: 25 mM; F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F3-Ca-a: 0.001 mM; F4-Ca-b: 0.01 mM; F5-Ca-c: 0.1 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
			SF12-F1-Ctrl-A				
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
			SF12-F2-ctrl-B				
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
			F7-ca-1				
0	8:55	0	0.00	0.167	1	0.100	
1	10:25	90	1.50	0.168	1	0.101	0.101
2	11:35	160	2.67	0.172	1	0.105	0.105
3	12:55	240	4.00	0.171	1	0.104	0.104
4	14:15	320	5.33	0.171	1	0.104	
5	8:30	1395	23.25	0.144	1	0.077	
			F8-ca-2				
0	8:55	0	0.00	0.178	1	0.111	
1	10:25	90	1.50	0.179	1	0.112	0.112
2	11:35	160	2.67	0.175	1	0.108	0.108
3	12:55	240	4.00	0.174	1	0.107	0.107
4	14:15	320	5.33	0.170	1	0.103	0.103
5	8:30	1395	23.25	0.136	1	0.069	
			F9-ca-3				
0	8:55	0	0.00	0.175	1	0.108	
1	10:25	90	1.50	0.179	1	0.112	0.112
2	11:35	160	2.67	0.173	1	0.106	0.106
3	12:55	240	4.00	0.169	1	0.102	0.102
4	14:15	320	5.33	0.160	1	0.093	0.093
5	8:30	1395	23.25	0.122	1	0.055	
			SF13-F1-ctrl-A				
0	8:15	0	0.00	0.160	1	0.093	
1	9:45	90	1.50	0.169	1	0.102	
2	11:05	170	2.83	0.229	1	0.162	0.162
3	12:20	245	4.08	0.368	1	0.301	0.301
4	13:35	320	5.33	0.215	5	0.752	0.752
6	8:30	1455	24.25	0.644	20	11.597	
			SF13 - F2-ctrl-B				
0	8:15	0	0.00	0.159	1	0.092	
1	9:45	90	1.50	0.168	1	0.101	
2	11:05	170	2.83	0.228	1	0.161	0.161
3	12:20	245	4.08	0.372	1	0.305	0.305
4	13:35	320	5.33	0.215	5	0.752	0.752
5	8:30	1455	24.25	0.652	20	11.757	
			F3-Ca-1				
0	8:15	0	0.00	0.165	1	0.098	
1	9:45	90	1.50	0.164	1	0.097	0.097
2	11:05	170	2.83	0.165	1	0.098	0.098
3	12:20	245	4.08	0.167	1	0.100	0.100
4	13:35	320	5.33	0.167	1	0.100	0.100
5	8:30	1455	24.25	0.167	1	0.100	0.100
			F4-Ca-10				
0	8:15	0	0.00	0.167	1	0.100	
1	9:45	90	1.50	0.169	1	0.102	0.102

TABLE 11-continued

Data for control samples and cinnamaldehyde-inhibited experiments. Cinnamaldehyde concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F7-ca-1: 50 mM; F8-ca-2: 100 mM; F9-ca-3: 200 mM; SF13-F1-ctrl-A: 0 mM; SF13-F2-ctrl-B: 0 mM; F3-Ca-1: 1 mM; F4-Ca-10: 10 mM; F5-Ca-25: 25 mM; F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F3-Ca-a: 0.001 mM; F4-Ca-b: 0.01 mM; F5-Ca-c: 0.1 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
2	11:05	170	2.83	0.170	1	0.103	0.103
3	12:20	245	4.08	0.174	1	0.107	0.107
4	13:35	320	5.33	0.172	1	0.105	0.105
5	8:30	1455	24.25	0.141	1	0.074	0.074
			F5-Ca-25				
0	8:15	0	0.00	0.171	1	0.104	
1	9:45	90	1.50	0.173	1	0.106	0.106
2	11:05	170	2.83	0.172	1	0.105	0.105
3	12:20	245	4.08	0.174	1	0.107	0.107
4	13:35	320	5.33	0.168	1	0.101	0.101
5	8:30	1455	24.25	0.140	1	0.073	0.073
			F1-Ctrl-A				
0	8:25	0	0.00	0.167	1	0.100	
1	9:55	90	1.50	0.190	1	0.123	
2	11:15	170	2.83	0.264	1	0.197	0.197
3	12:35	250	4.17	0.452	1	0.385	0.385
4	13:40	315	5.25	0.246	5	0.907	0.907
			F2-ctrl-B				
0	8:25	0	0.00	0.165	1	0.098	
1	9:55	90	1.50	0.190	1	0.123	
2	11:15	170	2.83	0.264	1	0.197	0.197
3	12:35	250	4.17	0.460	1	0.393	0.393
4	13:40	315	5.25	0.248	5	0.917	0.917
			F3-Ca-a				
0	8:25	0	0.00	0.166	1	0.099	
1	9:55	90	1.50	0.192	1	0.125	
2	11:15	170	2.83	0.266	1	0.199	0.199
3	12:35	250	4.17	0.461	1	0.394	0.394
4	13:40	315	5.25	0.256	5	0.957	0.957
			F4-Ca-b				
0	8:25	0	0.00	0.169	1	0.102	
1	9:55	90	1.50	0.190	1	0.123	
2	11:15	170	2.83	0.262	1	0.195	0.195
3	12:35	250	4.17	0.443	1	0.376	0.376
4	13:40	315	5.25	0.245	5	0.902	0.902
			F5-Ca-c				
0	8:25	0	0.00	0.164	1	0.097	
1	9:55	90	1.50	0.186	1	0.119	
2	11:15	170	2.83	0.235	1	0.168	0.168
3	12:35	250	4.17	0.364	1	0.297	0.297
4	13:40	315	5.25	0.575	1	0.508	0.508
5	14:55	390	6.50	0.321	5	1.282	1.282

Example 19

Inhibition of Ethanologen Yeast PNY 827 by 1-bromo-2-butanone

The inhibitory effect of 1-bromo-2-butanone on ethanologen yeast PNY 827 was investigated. Therefore a 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827. The culture was inoculated overnight at 30° C. and 250 rpm in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). Subsequently, a sufficient amount of the seed culture was transferred into shake flasks containing 20 ml of production medium without 1-bromo-2-butanone or addition of 1-bromo-2-butanone at concen-

trations of 50 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, 0.01 mM and 0.001 mM, to give an initial OD of approximately 0.1. The cultures were incubated at 250 rpm for 24 h in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) and samples of about 1 ml for OD determination withdrawn at designated hours. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. In case cell dry weight concentrations were needed, an OD-DW-correlation of 0.33 gDW/OD was applied. Maximum specific growth rates μ_{max} were determined by applying the exponential regression function of Microsoft Excel (Microsoft Office Excel 2003, SP 3). Outliers were discarded until good fit of the regression curve with measurements was confirmed by visual inspection. Parameters of the inhibition kinetics were determined by least square minimization of the differences between measured and predicted μ_{max} values. Employed search algorithm was a quasi-Newton method with linear extrapolation from a tangent vector, as implemented in the solver routine of Microsoft Excel (Microsoft Office Excel 2003, SP 3).

The inhibitory effect of 1-bromo-2-butanone was investigated at 50 mM, 5 mM, 1 mM, 0.5 mM, 0.1 mM, 0.01 mM and 0.001 mM. For generating the concentrations of 0.1 mM, 0.01 mM and 0.001 mM, 1-bromo-2-butanone was diluted with DMSO, resulting in DMSO concentrations in the cell suspension of 0.7 mM, 7 mM and 70 mM of DMSO, respectively. Assuming an additive effect of 1-bromo-2-butanone and DMSO inhibition, observed maximum specific growth rates of the two cultures were corrected by 0.000 l/h, 0.000 l/h and 0.005 l/h due to the effect of DMSO, resulting in 1-bromo-2-butanone-based mumax values of 0.54 l/h, 0.00 l/h and 0.00 l/h derived from the observed values of 0.54 l/h, 0.00 l/h and 0.00 l/h, respectively. At all the other concentrations, no DMSO was used for dilution of 1-bromo-2-butanone. However, in all of the non-DMSO experiments no cell growth was observed. Fitting the data to the "squared inhibition" kinetics (observed maximum specific growth rates without inhibitor addition were 0.59 l/h, 0.59 l/h, 0.60 l/h, 0.62 l/h, 0.62 l/h and 0.62 l/h) yielded parameters of μ°_{max}=0.61 l/h and a K_I value of K_I=0.002 mM (FIG. **13**). This corresponds to an IC50 value of 1-bromo-2-butanone on growth of 0.002 mM, indication of strong inhibition of ethanologen yeast by 1-bromo-2-butanone. Data from the samples is seen in Table 12 below.

TABLE 12

Data for control samples and 1-bromo-2-butanone-inhibited experiments, 1-bromo-2-butanone concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F10-bb-1: 5 mM; F11-bb-2: 50 mM; SF13-F1-ctrl-A: 0 mM; SF13-F2-ctrl-B: 0 mM; F6-Bb-0.5: 0.5 mM; F7-Bb-1: 1 mM; F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F6-Bb-a: 0.001 mM; F7-Bb-b: 0.01 mM; F8-Bb-c: 0.1 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
SF12-F1-Ctrl-A							
0	8:55	0	0.00	0.156	1	0.089	
1	10:25	90	1.50	0.170	1	0.103	
2	11:35	160	2.67	0.222	1	0.155	0.155
3	12:55	240	4.00	0.350	1	0.283	0.283
4	14:15	320	5.33	0.220	5	0.777	0.777
6	8:30	1395	23.25	0.648	20	11.677	
SF12-F2-ctrl-B							
0	8:55	0	0.00	0.162	1	0.095	
1	10:25	90	1.50	0.172	1	0.105	
2	11:35	160	2.67	0.223	1	0.156	0.156

TABLE 12-continued

Data for control samples and 1-bromo-2-butanone-inhibited experiments, 1-bromo-2-butanone concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F10-bb-1: 5 mM; F11-bb-2: 50 mM; SF13-F1-ctrl-A: 0 mM; SF13-F2-ctrl-B: 0 mM; F6-Bb-0.5: 0.5 mM; F7-Bb-1: 1 mM; F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F6-Bb-a: 0.001 mM; F7-Bb-b: 0.01 mM; F8-Bb-c: 0.1 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
3	12:55	240	4.00	0.354	1	0.287	0.287
4	14:15	320	5.33	0.228	5	0.817	0.817
5	8:30	1395	23.25	0.667	20	12.057	
F10-bb-1							
0	8:55	0	0.00	0.161	1	0.094	
1	10:25	90	1.50	0.176	1	0.109	0.109
2	11:35	160	2.67	0.170	1	0.103	0.103
3	12:55	240	4.00	0.168	1	0.101	0.101
4	14:15	320	5.33	0.170	1	0.103	0.103
5	8:30	1395	23.25	0.171	1	0.104	0.104
F11-bb-2							
0	8:55	0	0.00	0.261	1	0.194	
1	10:25	90	1.50	0.238	1	0.171	0.171
2	11:35	160	2.67	0.275	1	0.208	0.208
3	12:55	240	4.00	0.266	1	0.199	0.199
4	14:15	320	5.33	0.264	1	0.197	0.197
5	8:30	1395	23.25	0.161	1	0.094	0.094
SF13-F1-ctrl-A							
0	8:15	0	0.00	0.160	1	0.093	
1	9:45	90	1.50	0.169	1	0.102	
2	11:05	170	2.83	0.229	1	0.162	0.162
3	12:20	245	4.08	0.368	1	0.301	0.301
4	13:35	320	5.33	0.215	5	0.752	0.752
6	8:30	1455	24.25	0.644	20	11.597	
SF13 - F2-ctrl-B							
0	8:15	0	0.00	0.159	1	0.092	
1	9:45	90	1.50	0.168	1	0.101	
2	11:05	170	2.83	0.228	1	0.161	0.161
3	12:20	245	4.08	0.372	1	0.305	0.305
4	13:35	320	5.33	0.215	5	0.752	0.752
5	8:30	1455	24.25	0.652	20	11.757	
F6-Bb-0.5							
0	8:15	0	0.00	0.165	1	0.098	
1	9:45	90	1.50	0.166	1	0.099	0.099
2	11:05	170	2.83	0.169	1	0.102	0.102
3	12:20	245	4.08	0.169	1	0.102	0.102
4	13:35	320	5.33	0.167	1	0.100	0.100
5	8:30	1455	24.25	0.169	1	0.102	0.102
F7-Bb-1							
0	8:15	0	0.00	0.157	1	0.090	
1	9:45	90	1.50	0.161	1	0.094	0.094
2	11:05	170	2.83	0.160	1	0.093	0.093
3	12:20	245	4.08	0.160	1	0.093	0.093
4	13:35	320	5.33	0.158	1	0.091	0.091
5	8:30	1455	24.25	0.161	1	0.094	0.094
F1-Ctrl-A							
0	8:25	0	0.00	0.167	1	0.100	
1	9:55	90	1.50	0.190	1	0.123	
2	11:15	170	2.83	0.264	1	0.197	0.197
3	12:35	250	4.17	0.452	1	0.385	0.385
4	13:40	315	5.25	0.246	5	0.907	0.907
F2-ctrl-B							
0	8:25	0	0.00	0.165	1	0.098	
1	9:55	90	1.50	0.190	1	0.123	
2	11:15	170	2.83	0.264	1	0.197	0.197
3	12:35	250	4.17	0.460	1	0.393	0.393
4	13:40	315	5.25	0.248	5	0.917	0.917
F6-Bb-a							
0	8:25	0	0.00	0.165	1	0.098	
1	9:55	90	1.50	0.180	1	0.113	

TABLE 12-continued

Data for control samples and 1-bromo-2-butanone-inhibited experiments, 1-bromo-2-butanone concentrations in the experiments were: SF12-F1-Ctrl-A: 0 mM; SF12-F2-ctrl-B: 0 mM; F10-bb-1: 5 mM; F11-bb-2: 50 mM; SF13-F1-ctrl-A: 0 mM; SF13-F2-ctrl-B: 0 mM; F6-Bb-0.5: 0.5 mM; F7-Bb-1: 1 mM; F1-Ctrl-A: 0 mM; F2-ctrl-B: 0 mM; F6-Bb-a: 0.001 mM; F7-Bb-b: 0.01 mM; F8-Bb-c: 0.1 mM.

sample	time	time [min]	time [h]	OD600	dilution [1:x]	OD600corr [ ]	OD600corr [ ]
2	11:15	170	2.83	0.219	1	0.152	0.152
3	12:35	250	4.17	0.314	1	0.247	0.247
4	13:40	315	5.25	0.500	1	0.433	0.433
5	14:55	390	6.50	0.287	5	1.112	1.112
				F7-Bb-b			
0	8:25	0	0.00	0.166	1	0.099	
1	9:55	90	1.50	0.184	1	0.117	0.117
2	11:15	170	2.83	0.183	1	0.116	0.116
3	12:35	250	4.17	0.189	1	0.122	0.122
4	13:40	315	5.25	0.186	1	0.119	0.119
5	14:55	390	6.50	0.191	1	0.124	0.124
				F8-Bb-c			
0	8:25	0	0.00	0.164	1	0.097	
1	9:55	90	1.50	0.168	1	0.101	0.101
2	11:15	170	2.83	0.166	1	0.099	0.099
3	12:35	250	4.17	0.170	1	0.103	0.103
4	13:40	315	5.25	0.170	1	0.103	0.103
5	14:55	390	6.50	0.170	1	0.103	

Example 20

Effect of Ethanol Dehydrogenase and Pyruvate Decarboxylase Inhibitors on Growth and Product Formation of Mixed Cultures of Ethanologen and Butanologen Yeast

Effects of addition of ethanol dehydrogenase and pyruvate decarboxylase inhibitors on mixed cultures of ethanologen *S. cerevisiae* PNY 827 and the butanologen yeast *S. cerevisiae* PNY 2129 were investigated. Therefore two 125 ml aerobic shake flask were prepared with 20 ml SEED medium and each inoculated with 1 ml of frozen glycerol stock culture of PNY 2129 in the morning. Another 125 ml aerobic shake flask was prepared with 20 ml SEED medium and inoculated with 1 ml of frozen glycerol stock culture of PNY 827 in the afternoon. All cultures were incubated at 30° C. and 250 rpm overnight in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.). In the morning, sufficient seed culture volume of each strain to give OD600 of 1.000 in the resuspended solution was separately transferred into 50 mL sterile centrifuge tubes and spun down at 9500 rpm for 20 min in an Eppendorf Centrifuge 5804R (Eppendorf, Hamburg, Germany). Supernatants were discarded and the cell pellets resuspended in 20 ml of 0.9% NaCl solution. Optical density was measured with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at λ=600 nm. Subsequently "production" cultures were prepared in 25 ml Balch tubes by adding into each tube 6 ml Yeast synthetic w/o aa, w/o glucose, w/o ethanol, w/o Tween (2×), 1.2 ml supplement amino acid solution without histidine and uracil (SAAS-2 10×), 1.92 ml of 250 g/l glucose (ca. 40 g/l glucose) and 2.3 µl of 3M sodium acetate, as well as a specific amount of inoculum solutions, inhibitor solution and water according to the schema in Table 13:

TABLE 13

Schema showing strains and inhibitors solutions.

	inoculum solution		stock:	inhibitor solution		
				300 mM	2 mM (200 mM)	250 mM (2500 mM)
	PNY2129 [ul]	PNY827 [ul]	H2O [ul]	Py [ul]	Bb [ul]	Ca [ul]
T1-ctrl1 B	1200		1200			
T2-ctrl2 B	1200		1200			
T3-ctrl1 E		1200	1200			
T4-ctrl2 E		1200	1200			
T5-1:1-PyA	600	600	1080	120		
T6-1:1-PyB	600	600		1200		
T7-11:1-PyA	1100	100	1080	120		
T8-11:1-PyB	1100	100		1200		
T9-1:1-BbA	600	600	1188		12	
T10-1:1-BbB	600	600	1188		12	
T11-11:1-BbA	1100	100	1188		12	
T12-11:1-BbB	1100	100	1188		12	
T13-1:1-CaA	600	600	1188			12
T14-1:1-CaB	600	600	1080			120
T15-11:1-CaA	1100	100	1188			12
T16-11:1-CaB	1100	100	1080			120
T17-1:1-ctrl1	600	600	1200			
T18-11:1-ctrl1	1100	100	1200			

Inhibitor solutions were trans-cinnamaldehyde (Aldrich, #239968, CAS: 14371-10-9) dissolved in water either ad 250 mM or 2500 mM, 1-bromo-2-butanone (Sigma-Aldrich, #243299, CAS: 816-40-0) dissolved in water either at 2 mM or 200 mM, and pyrazole (Aldrich, # P56607, CAS Number: 288-13-1), dissolved in water at 300 mM. Resulting inhibitor concentrations in the Balch tube cultures were pyrazole (PY): 3 mM (A) and 30 mM (B), 1-bromo-2-butanone (BB): 2 µM (A) and 200 µM (B), and trans-cinnamaldehyde (CA): 250 µM (A) and 25 mM (B), respectively. Each Balch tube was fitted with a butyl rubber septum and crimped to the tube with a sheet metal with circular opening to allow samples withdrawal by syringes. The cultures were mixed by a vial/tube rotator (Glas-Col, Terre-Haute, Ind.) that was placed in an Innova Laboratory Shaker (New Brunswick Scientific, Edison, N.J.) for keeping the temperature at 30° C. Samples of about 1 ml for OD determination and extracellular compound analysis were withdrawn at designated hours. Extracellular compound analysis in supernatant was accomplished by HPLC. An Aminex® HPX-87H column (Bio-Rad, Hercules, Calif.) was used in an isocratic method with 0.01N sulfuric acid as eluent on an Alliance® 2695 Separations Module (Waters Corp., Milford, Mass.). Flow rate was 0.60 mL/min, column temperature 40° C., injection volume 10 µL and run time 58 min. Detection was carried out with a refractive index detector (Waters 2414 RI, Waters Corp., Milford, Mass.) operated at 40° C. and an UV detector (Waters 2996 PDA, Waters Corp., Milford, Mass.) at 210 nm. Determined optical densities as well as concentrations of extracellular compounds at selected sampling time points can be found in Table 14.

Butanol to ethanol formed in the mixed cultures with inhibitors was compared to the ratio of butanol to ethanol formed in the mixed cultures without inhibitor (Ctrl) at 8 hours (EPT=8 h, FIG. **14** and FIG. **15**) and at **48** hours (EPT=48 h, FIG. **16** and FIG. **17**) of the experiments inoculated with a butanologen-to-ethanologen ratio of 11:1 (b:e=11:1, FIG. **14** and FIG. **16**) or 1:1 (b:e=1:1, FIG. **15** and FIG. **17**).

No growth in both mixed cultures and at both time points was observed at the high concentration of trans-cinnamaldehyde of 25 mM (FIG. **14**-FIG. **17**). At the lower concentration of 250 μM, both mixed cultures grew and produced alcohols. However, at both sampling times (EPT=8 h and EPT=48 h) as well as at both inoculum ratios (1:1 and 1:11), the ratio of butanol vs. ethanol produced was lower with addition of trans-cinnamaldehyde than without addition (FIG. **14**-FIG. **17**).

With 1-bromo-2-butanone, no growth was observed in the 1:1 culture at the high concentration of 200 μM until EPT=8 h, only at EPT=48 h. At low concentration (2 μM), cultures with both inoculum ratios showed increased butanol-to-ethanol ratios at EPT=8 h, but not at EPT=48 h. The same findings apply to the culture with 1:11 ratio at the high concentration (FIG. **14**-FIG. **17**).

With pyrazole addition at both concentrations, 3 mM and 30 mM, cultures with inoculum ratios 1:1 as well as 1:11 showed dramatically increased butanol-to-ethanol ratios at EPT=8 h (FIG. **14** and FIG. **15**). However, at EPT=48 h cultures with both inoculum ratios maintained significantly increased butanol:ethanol ratios only at the higher pyrazole concentration of 30 mM, but not at the lower concentration of 3 mM (FIG. **16** and FIG. **17**).

TABLE 14

Optical density (OD) and extracellular compound concentrations at the different sampling time points (EPT = elapsed process time) of different pure and mixed cultures. Abbreviations used were: EtOH = ethanol, PYR = pyruvate, KTV = ketoisovalerate, DHIV = dihydroisovalerate, DHMB = 2,3-dihydroxy-2-metylbutyrate, GLY = glycerol, ACE = acetate, IBOOH = isobutyric acid, IBOH = isobutanol, m-BDO = meso-butanediol, d/l-BDO = d/l-butanediol, LAC = lactate, SUC = succinate.

Sample [ ]	EPT [h]	OD [ ]	GLC [mM]	EtOH [mM]	PYR [mM]	KIV [mM]	DHIV + DHMB [mM]	GLY [mM]	ACE [mM]	IBOOH [mM]	IBOH [mM]	m-BDO [mM]	d/l-BDO [mM]	LAC [mM]	SUC [mM]
SF-17-T1-ctrl-1-B-0	0.00	0.115	226.0	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T1-ctrl-1-B-3	3.00	0.159	223.4	0.0	0.0	0.2	0.0	0.2	6.7	0.0	0.6	0.0	0.0	0.0	0.0
SF-17-T1-ctrl-1-B-6	6.00	0.210	221.3	0.0	0.1	0.2	0.0	0.3	6.7	0.3	1.4	0.0	0.0	0.0	0.0
SF-17-T1-ctrl-1-B-8	8.00	0.247	222.4	0.0	0.1	0.4	0.0	0.4	6.5	0.4	2.4	0.0	0.0	0.0	0.0
SF-17-T1-ctrl-1-B-24	24.00	1.382	178.0	1.2	1.8	3.1	0.6	2.8	4.4	2.5	24.8	0.0	0.0	0.1	0.3
SF-17-T1-ctrl-1-B-31	31.00	1.747	147.9	2.0	2.2	3.8	1.2	5.2	4.1	3.1	39.7	0.0	0.5	0.1	0.5
SF-17-T1-ctrl-1-B-48	48.00	1.917	106.9	3.6	2.9	4.4	1.9	11.9	3.0	3.3	69.1	0.0	1.3	0.2	0.5
SF-17-T1-ctrl-1-B-0	0.00	0.109	226.0	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T2-ctrl-2-B-3	3.00	0.157	223.4	0.0	0.0	0.1	0.0	0.2	6.7	0.0	0.6	0.0	0.0	0.0	0.0
SF-17-T2-ctrl-2-B-6	6.00	0.209	222.0	0.0	0.1	0.2	0.0	0.3	6.7	0.2	1.4	0.0	0.0	0.0	0.0
SF-17-T2-ctrl-2-B-8	8.00	0.239	221.1	0.0	0.1	0.4	0.0	0.4	6.5	0.3	2.4	0.0	0.0	0.0	0.0
SF-17-T2-ctrl-2-B-24	24.00	1.197	183.6	0.0	1.6	2.9	0.4	2.6	4.4	2.9	22.2	0.0	0.0	0.0	0.2
SF-17-T2-ctrl-2-B-31	31.00	1.627	152.0	2.1	2.1	3.9	1.1	4.9	4.0	3.3	38.1	0.0	0.5	0.0	0.5
SF-17-T2-ctrl-2-B-48	48.00	1.867	104.7	4.0	3.0	4.6	2.0	11.2	2.9	3.3	69.7	0.0	1.4	0.3	0.5
SF-17-T3-ctrl-1-E-0	0.00	0.088	224.0	0.0	0.0	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T3-ctrl-1-E-3	3.00	0.222	221.8	4.5	0.0	0.0	0.0	0.4	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T3-ctrl-1-E-6	6.00	1.317	206.1	30.3	0.1	0.0	0.0	1.6	6.6	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T3-ctrl-1-A-8	8.00	3.637	162.9	103.8	0.5	0.0	0.0	5.7	6.3	0.0	0.0	0.0	0.2	0.0	0.3
SF-17-T3-ctrl-1-E-24	24.00	9.497	0.0	378.5	2.5	0.0	0.0	18.6	9.5	0.0	0.0	0.0	0.5	0.4	0.5
SF-17-T3-ctrl-1-E-31	31.00	11.997	0.0	372.7	2.6	0.0	0.0	18.6	10.8	0.0	0.5	0.0	0.4	0.0	0.5
SF-17-T3-ctrl-1-E-48	48.00	11.897	0.0	382.0	2.3	0.0	0.0	18.6	11.0	0.0	0.0	0.0	0.3	0.3	0.6
SF-17-T3-ctrl-1-E-0	0.00	0.088	224.0	0.0	0.0	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T4-ctrl-2-E-3	3.00	0.225	221.7	4.6	0.0	0.0	0.0	0.4	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T4-ctrl-2-E-6	6.00	1.352	207.3	30.6	0.1	0.0	0.0	1.6	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T4-ctrl-1-B-8	8.00	3.727	161.8	104.8	0.5	0.0	0.0	5.6	6.4	0.0	0.0	0.0	0.0	0.2	0.3
SF-17-T4-ctrl-2-E-24	24.00	11.847	0.0	377.1	2.5	0.0	0.0	17.0	12.1	0.0	0.0	0.0	0.4	0.4	0.5
SF-17-T4-ctrl-2-E-31	31.00	11.547	0.0	367.9	2.4	0.0	0.0	17.1	13.0	0.0	0.6	0.0	0.4	0.4	0.5
SF-17-T4-ctrl-2-E-48	48.00	11.747	0.0	378.7	2.3	0.0	0.0	16.9	13.6	0.0	0.5	0.0	0.3	0.3	0.4
SF-17-T5-1:1-Py-A-0	0.00	0.104	224.0	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T5-1:1-Py-A-3	3.00	0.141	222.8	1.3	0.0	0.0	0.0	0.7	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T5-1:1-Py-A-6	6.00	0.273	218.1	5.4	0.1	0.1	0.0	2.5	6.9	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T5-1:1-Py-A-8	8.00	0.504	210.2	14.8	0.2	0.1	0.0	6.4	6.8	0.0	0.6	0.4	0.0	0.0	0.0
SF-17-T5-1:1-Py-A-24	24.00	9.777	0.0	346.4	2.9	0.1	0.0	37.0	10.3	0.1	1.6	0.9	1.5	0.4	0.4
SF-17-T5-1:1-Py-A-31	31.00	10.647	0.0	337.3	2.8	0.1	0.0	37.0	11.7	0.3	1.6	0.9	1.6	0.3	0.4
SF-17-T5-1:1-Py-A-48	48.00	10.797	0.0	347.2	2.7	0.1	0.0	37.1	12.7	0.0	1.6	0.9	1.7	0.3	0.5
SF-17-T5-1:1-Py-A-0	0.00	0.103	224.0	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T6-1:1-Py-B-3	3.00	0.125	222.4	0.0	0.0	0.0	0.0	0.7	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T6-1:1-Py-B-6	6.00	0.149	220.7	1.4	0.0	0.1	0.0	2.0	6.8	0.0	0.0	0.2	0.0	0.0	0.0
SF-17-T6-1:1-Py-B-8	8.00	0.173	218.7	2.4	0.1	0.1	0.0	3.9	7.0	0.0	0.2	0.3	0.0	0.0	0.0
SF-17-T6-1:1-Py-B-24	24.00	0.602	175.8	24.6	2.0	0.3	0.0	32.7	7.5	0.0	2.1	2.5	0.0	0.0	0.0
SF-17-T6-1:1-Py-B-31	31.00	0.727	149.4	46.0	3.8	0.4	0.0	48.4	8.2	0.0	2.5	3.2	0.7	0.0	0.0
SF-17-T6-1:1-Py-B-48	48.00	1.477	68.8	140.3	9.1	0.5	0.0	88.0	10.0	0.0	3.0	3.8	2.0	0.0	0.6
SF-17-T8-11:1-Py-B-0	0.00	0.110	223.6	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T7-11:1-Py-A-3	3.00	0.146	223.6	0.0	0.0	0.1	0.0	0.3	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T7-11:1-Py-A-6	6.00	0.174	224.2	0.9	0.0	0.1	0.0	0.8	6.7	0.3	0.4	0.0	0.0	0.0	0.0
SF-17-T7-11:1-Py-A-8	8.00	0.216	221.3	2.5	0.1	0.2	0.0	1.6	6.6	0.4	0.7	0.0	0.0	0.0	0.0
SF-17-T7-11:1-Py-A-24	24.00	9.517	0.0	345.0	3.0	0.3	0.0	37.3	8.8	0.8	3.4	0.0	1.2	0.4	0.5
SF-17-T7-11:1-Py-A-31	31.00	10.047	0.0	336.0	2.9	0.3	0.0	37.4	10.1	0.8	3.4	0.9	1.4	0.4	0.5
SF-17-T7-11:1-Py-A-48	48.00	11.347		344.2	2.8	0.2	0.0	37.2	11.4	0.6	3.5	0.9	1.4	0.3	0.5
SF-17-T8-11:1-Py-B-0	0.00	0.108	223.6	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T8-11:1-Py-B-3	3.00	0.136	223.2	0.0	0.0	0.1	0.0	0.3	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T8-11:1-Py-B-6	6.00	0.150	224.5	0.4	0.0	0.2	0.0	0.7	6.7	0.3	0.2	0.0	0.0	0.0	0.0
SF-17-T8-11:1-Py-B-8	8.00	0.161	222.1	0.7	0.0	0.2	0.0	1.1	6.8	0.3	0.2	0.0	0.0	0.0	0.0
SF-17-T8-11:1-Py-B-24	24.00	0.277	209.8	6.0	0.4	0.6	0.0	6.6	6.6	0.8	1.8	0.0	0.0	0.0	0.0
SF-17-T8-11:1-Py-B-31	31.00	0.327	205.2	10.2	0.6	0.8	0.0	10.7	6.7	1.1	3.6	0.3	0.0	0.0	0.0

TABLE 14-continued

Optical density (OD) and extracellular compound concentrations at the different sampling time points (EPT = elapsed process time) of different pure and mixed cultures. Abbreviations used were: EtOH = ethanol, PYR = pyruvate, KTV = ketoisovalerate, DHIV = dihydroisovalerate, DHMB = 2,3-dihydroxy-2-metylbutyrate, GLY = glycerol, ACE = acetate, IBOOH = isobutyric acid, IBOH = isobutanol, m-BDO = meso-butanediol, d/l-BDO = d/l-butanediol, LAC = lactate, SUC = succinate.

Sample [ ]	EPT [h]	OD [ ]	GLC [mM]	EtOH [mM]	PYR [mM]	KIV [mM]	DHIV + DHMB [mM]	GLY [mM]	ACE [mM]	IBOOH [mM]	IBOH [mM]	m-BDO [mM]	d/l-BDO [mM]	LAC [mM]	SUC [mM]
SF-17-T8-11:1-Py-B-48	48.00	0.667	164.4	31.3	2.0	1.3	0.0	29.7	7.3	2.0	12.1	1.2	0.0	0.0	0.0
SF-17-T9-1:1-Bb-A-0	0.00	0.104	224.2	0.0	0.0	0.0	0.0	0.1	6.9	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T9-1:1-Bb-A-3	3.00	0.144	223.2	1.4	0.0	0.1	0.0	0.2	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T9-1:1-Bb-A-6	6.00	0.330	219.1	7.3	0.0	0.1	0.0	0.7	6.6	0.0	0.4	0.0	0.0	0.0	0.0
SF-17-T9-1:1-Bb-A-8	8.00	1.057	207.4	26.7	0.1	0.1	0.0	2.0	6.6	0.2	0.7	0.0	0.0	0.0	0.0
SF-17-T9-1:1-Bb-A-24	24.00	9.877	0.0	371.9	2.7	0.2	0.0	20.5	9.9	0.5	2.0	0.0	0.3	0.3	0.5
SF-17-T9-1:1-Bb-A-31	31.00	11.047	0.0	363.4	2.6	0.2	0.0	20.5	10.9	0.7	2.0	0.0	0.3	0.3	0.5
SF-17-T9-1:1-Bb-A-48	48.00	12.247	0.0	375.3	2.5	0.2	0.0	20.4	11.8	0.7	2.0	0.4	0.4	0.3	0.6
SF-17-T9-1:1-Bb-A-0	0.00	0.106	224.2	0.0	0.0	0.0	0.0	0.1	6.9	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T10-1:1-Bb-B-3	3.00	0.114	223.7	0.0	0.0	0.0	0.0	0.2	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T10-1:1-Bb-B-6	6.00	0.139	223.4	1.4	0.0	0.0	0.0	0.3	6.8	0.0	0.2	0.0	0.0	0.0	0.0
SF-17-T10-1:1-Bb-B-8	8.00	0.189	223.8	3.8	0.0	0.1	0.0	0.4	6.8	0.0	0.3	0.0	0.0	0.0	0.0
SF-17-T10-1:1-Bb-B-24	24.00	11.847	0.0	373.8	2.6	0.1	0.0	19.2	9.3	0.2	1.3	0.0	0.2	0.3	0.6
SF-17-T10-1:1-Bb-B-31	31.00	11.447	0.0	364.2	2.6	0.1	0.0	19.4	11.0	0.0	1.3	0.3	0.3	0.3	0.6
SF-17-T10-1:1-Bb-B-48	48.00	11.747	0.0	380.5	2.5	0.1	0.0	19.2	12.0	0.3	1.3	0.0	0.3	0.3	0.6
SF-17-T12-11:1-Bb-B-0	0.00	0.110	223.9	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T11-11:1-Bb-A-3	3.00	0.144	224.0	0.0	0.0	0.1	0.0	0.2	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T11-11:1-Bb-A-6	6.00	0.187	222.0	1.0	0.0	0.0	0.0	0.4	6.6	0.0	0.6	0.0	0.0	0.0	0.0
SF-17-T11-11:1-Bb-A-8	8.00	0.259	220.8	3.4	0.1	0.2	0.0	0.7	6.5	0.0	1.0	0.0	0.2	0.0	0.0
SF-17-T11-11:1-Bb-A-24	24.00	10.797	0.0	363.4	2.8	0.5	0.0	22.3	8.4	1.0	4.4	0.0	0.2	0.3	0.5
SF-17-T11-11:1-Bb-A-31	31.00	11.197	0.0	353.8	2.7	0.5	0.0	22.3	10.0	1.1	4.3	0.6	0.4	0.4	0.5
SF-17-T11-11:1-Bb-A-48	48.00	11.497	0.0	365.6	2.7	0.4	0.0	22.2	11.3	1.4	4.5	0.6	0.5	0.3	0.5
SF-17-T12-11:1-Bb-B-0	0.00	0.107	223.9	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T12-11:1-Bb-B-3	3.00	0.123	222.4	0.0	0.0	0.0	0.0	0.2	6.7	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T12-11:1-Bb-B-6	6.00	0.134	222.4	0.0	0.0	0.1	0.0	0.2	6.6	0.0	0.4	0.0	0.0	0.0	0.0
SF-17-T12-11:1-Bb-B-8	8.00	0.138	225.8	0.0	0.0	0.1	0.0	0.3	6.9	0.0	0.5	0.0	0.0	0.0	0.0
SF-17-T12-11:1-Bb-B-24	24.00	10.317	16.0	344.8	2.7	0.2	0.0	20.4	6.4	0.5	2.8	0.3	0.0	0.4	0.5
SF-17-T12-11:1-Bb-B-31	31.00	11.447	0.0	360.8	2.7	0.2	0.0	21.4	9.1	0.5	2.8	0.0	0.2	0.3	0.6
SF-17-T12-11:1-Bb-B-48	48.00	11.897	0.0	372.0	2.6	0.2	0.0	21.1	10.8	0.6	2.8	0.0	0.3	0.4	0.6
SF-17-T14-1:1-Ca-B-0	0.00	0.106	233.7	0.0	0.0	0.0	0.0	0.1	7.2	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T13-1:1-Ca-A-3	3.00	0.142	222.8	1.6	0.0	0.0	0.0	0.3	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T13-1:1-Ca-A-6	6.00	0.388	217.9	9.6	0.0	0.0	0.0	0.8	6.6	0.0	0.3	0.0	0.0	0.0	0.0
SF-17-T13-1:1-Ca-A-8	8.00	1.417	201.3	36.8	0.2	0.1	0.0	2.5	6.5	0.3	0.6	0.0	0.0	0.0	0.0
SF-17-T13-1:1-Ca-A-24	24.00	11.347	0.0	374.9	2.7	0.1	0.0	18.4	11.2	0.5	1.7	0.0	0.2	0.4	0.5
SF-17-T13-1:1-Ca-A-31	31.00	10.647	0.0	358.7	2.6	0.1	0.0	18.5	11.9	0.7	1.6	0.0	0.3	0.3	0.5
SF-17-T13-1:1-Ca-A-48	48.00	12.097	0.0	369.5	2.5	0.1	0.0	18.4	13.0	0.7	1.6	0.2	0.2	0.3	0.5
SF-17-T14-1:1-Ca-B-0	0.00	n.d.	233.7	0.0	0.0	0.0	0.0	0.1	7.2	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-3	3.00	n.d.	233.9	0.0	0.0	0.0	0.0	0.1	7.2	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-6	6.00	n.d.	233.4	0.0	0.0	0.0	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-8	8.00	n.d.	237.4	0.0	0.0	0.0	0.0	0.1	7.2	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-24	24.00	n.d.	234.0	0.0	0.0	0.0	0.0	0.0	7.2	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-31	31.00	n.d.	234.0	0.0	0.0	0.0	0.0	0.1	7.1	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T14-1:1-Ca-B-48	48.00	n.d.	234.7	0.0	0.0	0.0	0.0	0.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T15-11:1-Ca-A-0	0.00	0.113	232.1	0.0	0.0	0.0	0.0	0.1	7.0	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T15-11:1-Ca-A-3	3.00	0.141	231.6	0.0	0.0	0.0	0.0	0.2	7.0	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T15-11:1-Ca-A-6	6.00	0.199	230.2	1.4	0.0	0.1	0.0	0.5	6.8	0.0	0.4	0.0	0.0	0.0	0.0
SF-17-T15-11:1-Ca-A-8	8.00	0.387	228.2	6.5	0.1	0.2	0.0	1.0	6.8	0.6	0.8	0.0	0.0	0.0	0.0
SF-17-T15-11:1-Ca-A-24	24.00	11.647	0.0	381.6	2.9	0.3	0.0	22.4	9.3	1.1	3.5	0.4	0.3	0.3	0.5
SF-17-T15-11:1-Ca-A-31	31.00	10.397	0.0	366.1	2.9	0.3	0.0	22.6	10.7	1.5	3.5	0.4	0.2	0.4	0.5
SF-17-T15-11:1-Ca-A-48	48.00	12.147	0.0	382.7	2.8	0.3	0.0	22.2	11.9	1.3	3.7	0.0	0.3	0.3	0.5
SF-17-T15-11:1-Ca-A-0	0.00	n.d.	232.1	0.0	0.0	0.0	0.0	0.1	7.0	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-3	3.00	n.d.	224.4	0.0	0.0	0.0	0.0	0.1	6.9	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-6	6.00	n.d.	223.9	0.0	0.0	0.0	0.0	0.1	6.7	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-8	8.00	n.d.	227.0	0.0	0.0	0.0	0.0	0.1	7.1	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-24	24.00	n.d.	224.1	0.0	0.0	0.0	0.0	0.0	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-31	31.00	n.d.	226.4	0.0	0.0	0.0	0.0	0.1	6.9	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T16-11:1-Ca-B-48	48.00	n.d.	225.3	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T17-1:1-ctrl-1-0	0.00	0.101	223.9	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T17-1:1-ctrl-1-3	3.00	0.209	221.6	2.5	0.0	0.1	0.0	0.4	6.8	0.0	0.4	0.0	0.0	0.0	0.0
SF-17-T17-1:1-ctrl-1-6	6.00	0.862	211.4	17.9	0.1	0.1	0.0	1.3	6.5	0.0	0.8	0.0	0.0	0.0	0.0
SF-17-T17-1:1-ctrl-1-8	8.00	2.787	181.0	67.6	0.4	0.2	0.0	4.7	6.5	0.2	1.2	0.0	0.0	0.1	0.2
SF-17-T17-1:1-ctrl-1-24	24.00	12.097	0.0	372.5	2.7	0.2	0.0	19.6	9.7	0.3	2.2	0.0	0.2	0.4	0.5
SF-17-T17-1:1-ctrl-1-31	31.00	11.997	0.0	358.7	2.7	0.2	0.0	19.9	10.3	0.0	2.1	0.3	0.4	0.3	0.6
SF-17-T17-1:1-ctrl-1-48	48.00	12.297	0.0	374.6	2.6	0.2	0.0	19.4	11.0	0.3	2.3	0.0	0.4	0.3	0.6
SF-17-T17-1:1-ctrl-1-0	0.00	0.114	223.9	0.0	0.0	0.0	0.0	0.1	6.8	0.0	0.0	0.0	0.0	0.0	0.0
SF-17-T18-11:1-ctrl-1-3	3.00	0.177	222.1	0.0	0.0	0.1	0.0	0.2	6.7	0.0	0.7	0.0	0.0	0.0	0.0
SF-17-T18-11:1-ctrl-1-6	6.00	0.303	218.8	2.9	0.1	0.3	0.0	0.5	6.4	0.3	1.5	0.0	0.0	0.0	0.0
SF-17-T18-11:1-ctrl-1-8	8.00	0.857	212.3	12.6	0.2	0.4	0.0	1.4	6.4	0.5	2.5	0.0	0.0	0.0	0.0
SF-17-T18-11:1-ctrl-1-24	24.00	11.397	0.0	354.1	3.1	0.6	0.1	21.6	8.4	0.8	7.2	0.0	0.4	0.4	0.5

TABLE 14-continued

Optical density (OD) and extracellular compound concentrations at the different sampling time points (EPT = elapsed process time) of different pure and mixed cultures. Abbreviations used were: EtOH = ethanol, PYR = pyruvate, KTV = ketoisovalerate, DHIV = dihydroisovalerate, DHMB = 2,3-dihydroxy-2-metylbutyrate, GLY = glycerol, ACE = acetate, IBOOH = isobutyric acid, IBOH = isobutanol, m-BDO = meso-butanediol, d/l-BDO = d/l-butanediol, LAC = lactate, SUC = succinate.

Sample [ ]	EPT [h]	OD [ ]	GLC [mM]	EtOH [mM]	PYR [mM]	KIV [mM]	DHIV + DHMB [mM]	GLY [mM]	ACE [mM]	IBOOH [mM]	IBOH [mM]	m-BDO [mM]	d/l-BDO [mM]	LAC [mM]	SUC [mM]
SF-17-T18-11:1-ctrl-1-31	31.00	10.947	0.0	351.4	3.1	0.6	0.1	21.9	9.6	1.0	7.1	0.8	0.5	0.4	0.5
SF-17-T18-11:1-ctrl-1-48	48.00	11.797	0.0	355.5	3.0	0.6	0.1	21.4	10.5	0.8	7.3	0.9	0.7	0.3	0.5

Materials & Methods for Examples 21-25

Yeast synthetic medium w/o amino acids, w/o glucose, w/o ethanol/Tween (2×): 13.4 g/l, Yeast Nitrogen Base w/o amino acids (Difco 0919-15-3); 40 mg/L thiamine; 40 mg/L niacin; 200 ml/L 1M MES buffer, pH=5.5; Supplement aa sol. without histidine and uracil (SAAS-1 10×); 18.5 g/L, Synthetic complete amino acid dropout (Kaiser)-His, -Ura (Formedium).

SEED medium: 10.000 mL Yeast synthetic medium w/o aa, w/o glucose, w/o ethanol/Tween (2×); 2.000 mL Supplement aa sol. without histidine and uracil (SAAS-1 10×); 3.200 mL 250 g/L glucose solution (resulting in 40 g/l glucose); 0.046 mL Na-acetate stock solution; 4.754 mL H_2O

Example 21 (Prophetic):

Construction of Isobutanologen Strains Expressing a Formaldehyde Dehydrogenase

P. putida fdhA (SEQ ID NO:7) (GI:1169603) and *S. cerevisiae* SFA1 (SEQ ID NO:6) (van den Berg et al., *Yeast* 13(6): 551-9 (1997)) are used to synthesize genes in vitro using codon-optimization algorithms for *S. cerevisiae* (e.g. DNA 2.0). The gene cassettes are designed to place 5' BamHI and 3' MluI restriction sites for subcloning of the coding sequences into expression plasmid pBTX1 (SEQ ID NO:15). pBTX1 is derived from the pRS413 vector backbone (ATCC #87518) and contains the FBA1 promoter, multiple cloning site (BamHI, MluI), and ADH1 terminator.

An isobutanologen is constructed by transformation of plasmids pBTX1::SFA1 and pLH804::L2V4 into the host strain PNY2145. Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEF1(M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

An isobutanologen is constructed by transformation of plasmids pBTX1::fdhA and pLH804::L2V4 into the host strain PNY2145. Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEF1(M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

Example 22 (Prophetic):

Construction of Isobutanologen Strains Expressing a Sulfonylurea-Resistant ALS (e.g. SMR1-410)

To construct an expression plasmid, the protein coding sequence for *S. cerevisiae* SMR1-410 (SEQ ID NO:9; nucleic acid sequence SEQ ID NO:8) is used to synthesize genes in vitro using codon-optimization algorithms for *S. cerevisiae* (e.g. DNA 2.0). The SMR1-410 gene cassette is designed to place 5' BamHI and 3' MluI restriction sites for subcloning of the coding sequences into expression plasmid pBTX1 (SEQ ID NO:15). pBTX1 is derived from the pRS413 vector backbone (ATCC #87518) and contains the FBA1 promoter, multiple cloning site (BamHI, MluI), and ADH1 terminator.

An isobutanologen is constructed by transformation of plasmids pBTX1::SMR1-410 and pLH804::L2V4 into the host strain PNY2145 (referenced in US Pat. Publ. No. 2014/0004526, which is incorporated herein by reference in its entirety, and described in Example 26). Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K_9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEF1(M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

Example 23 (Prophetic):

Construction of Isobutanologen Strains Expressing Genes Conferring Sulfite Resistance

To construct expression plasmids, the protein coding sequences for *S. cerevisiae* FZF1-4 (SEQ ID NO:11) (Park,

Lopez et al. 1999) and SSU1 (SEQ ID NO:12) are used to synthesize genes in vitro using codon-optimization algorithms for *S. cerevisiae* (e.g. DNA 2.0). SEQ ID NO:10 is the wild type protein sequence for FZF1. The gene cassettes are designed to place 5' BamHI and 3' MluI restriction sites for subcloning of the coding sequences into expression plasmid pBTX1 (SEQ ID NO:15). pBTX1 is derived from the pRS413 vector backbone (ATCC #87518) and contains the FBA1 promoter, multiple cloning site (BamHI, MluI), and ADH1 terminator.

An isobutanologen is constructed by transformation of plasmids pBTX1::FZF1-4 and pLH804::L2V4 into the host strain PNY2145 (described herein) that contains a deletion of the chromosomal FZF1 gene. The FZF1 deletion in PNY2145 is made using standard yeast deletions using a kanMX4 cassette (Brachmann, et al. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast. 14, 115-132 (1998). Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEF1(M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

An isobutanologen is constructed by transformation of plasmids pBTX1::SSU/and pLH804::L2V4 into the host strain PNY2145 (described herein). Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEF1(M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

Example 24 (Prophetic):

Construction of Isobutanologen Strains Expressing a Glyphosate Resistance 3-phosphoshikimate 1-carboxylvinyltransferase

To construct an expression plasmid, the protein coding sequence for *Salmonella typhi* aroA^{GLY+} (SEQ ID NO:13) (Stalker, et al., *J Biol Chem* 260(8): 4724-8 (1985)) is used to synthesize genes in vitro using codon-optimization algorithms for *S. cerevisiae* (e.g. DNA 2.0). The aroA^{GLY+} gene cassette is designed to place 5' BamHI and 3' MluI restriction sites for subcloning of the coding sequences into expression plasmid pBTX1 (SEQ ID NO:15). pBTX1 is derived from the pRS413 vector backbone (ATCC #87518) and contains the FBA1 promoter, multiple cloning site (BamHI, MluI), and ADH1 terminator.

An isobutanologen is constructed by transformation of plasmids pBTX1::aroA^{GLY+} and pLH804::L2V4 into the host strain PNY2145 (described herein). Plasmid pLH804::L2V4 is derived from the pHR81 vector backbone (ATCC #87541) and contains: the *A. caccae* K9JB4P KARI driven by the ILV5 promoter and ILV5 terminator, and the *S. mutans* L2V4 DHAD driven by the TEFL (M7) promoter and FBA1 terminator (SEQ ID NO:22). Plasmids are introduced by lithium acetate transformation method (Methods in Yeast Genetics, 2005, page 113), and transformants are selected on synthetic complete medium, minus histidine and uracil, with 1% ethanol as carbon source. Transformants are then transferred to plates containing synthetic complete medium, minus histidine and uracil, with 2% glucose as carbon source and optionally ethanol (0.05%) or acetate (2 mM) as a C2 supplement. Freezer vials are made by dilution of log-phase cultures with 45% glycerol to a final glycerol concentration of 15% (w/v).

Example 25 (Prophetic):

Genetic Engineering for Increased Inhibitor Tolerance in Butanologen Yeast

In some embodiments, the butanologen is engineered for increased inhibitor tolerance by expressing or overexpressing a formaldehyde dehydrogenase. The formaldehyde dehydrogenase is selected from one of the following EC groups: EC 1.1.1.284, EC 1.1.1.1, EC 1.2.1.46, EC 1.2.1.66, EC 3.1.2.12, EC 1.2.2.B1 and EC 1.2.2.B2. ED 1.2.2.B1 and EC 1.2.2.B2 are no official designators, but are defined by the BRENDA protein database. Especially suited formaldehyde dehydrogenases are:

SFA1 (YDL168W, ADH5): glutathione-dependent formaldehyde dehydrogenase (van den Berg et al., *Yeast* 13(6): 551-9 (1997)) (SEQ ID NO:6) and *Pseudomonas putida* glutathione-independent formaldehyde dehydrogenase (SEQ ID NO:7).

In some embodiments, the butanologen is engineered for increased inhibitor tolerance by expressing or overexpressing a sulfonylurea-resistant ALS (e.g. SMR1-410) (Yadav et al., *Proc Natl Acad Sci USA* 83(12): 4418-22 (1986)) (SEQ ID NO:9).

In some embodiments, the butanologen is engineered for increased inhibitor tolerance by expressing or overexpressing other sulfonylurea-resistant ALS enzymes that qualify for (over)expression.

In some embodiments, the butanologen is engineered for increased inhibitor tolerance by expressing or overexpressing sulfite resistance by convert FZF1 (SEQ ID NO:10) to FZF1-4 (SEQ ID NO:11) (Park et al., *Curr Genet* 36(6): 339-44. (1999)) or overexpressing SSU1 (SEQ ID NO:12).

FZF1 (YGL254W, NRC299, RSU1 2, SUL1) is a transcription factor involved in sulfite metabolism, sole identified regulatory target is SSU1, overexpression suppresses sulfite-sensitivity of many unrelated mutants due to hyperactivation of SSU1.

Overexpression of SSU1 (YPL092W, LPG16). SSU1 is a plasma membrane sulfite pump involved in sulfite metabolism and required for efficient sulfite efflux. Homolog enzymes may be considered for overexpression as well to confer increased tolerance/improved competitiveness.

In some embodiments, the butanologen is engineered for increased inhibitor tolerance by expressing or overexpress-

ing a glyphosate resistance 3-phosphoshikimate 1-carboxylvinyltransferase (e.g. $aroA^{gly+}$) (SEQ ID NO:13) (Stalker et al., *J Biol Chem* 260(8): 4724-8 (1985)).

All other glyphosate resistant 3-phosphoshikimate 1-carboxyvinyltransferases qualify for expression or overexpression.

Example 26

Strain Construction

Construction of Strain PNY2115

Saccharomyces cerevisiae strain PNY0827 is used as the host cell for further genetic manipulation for PNY2115. PNY0827 refers to a strain derived from *Saccharomyces cerevisiae* which has been deposited at the ATCC under the Budapest Treaty on Sep. 22, 2011 at the American Type Culture Collection, Patent Depository 10801 University Boulevard, Manassas, Va. 20110-2209 and has the patent deposit designation PTA-12105.

Deletion of URA3 and Sporulation into Haploids

In order to delete the endogenous URA3 coding region, a deletion cassette was PCR-amplified from pLA54 (SEQ ID NO:158) which contains a P_{TEF1}-kanMX4-TEF1t cassette flanked by loxP sites to allow homologous recombination in vivo and subsequent removal of the KANMX4 marker. PCR was done by using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and primers BK505 (SEQ ID NO:101) and BK506 (SEQ ID NO:102). The URA3 portion of each primer was derived from the 5' region 180 bp upstream of the URA3 ATG and 3' region 78 bp downstream of the coding region such that integration of the kanMX4 cassette results in replacement of the URA3 coding region. The PCR product was transformed into PNY0827 using standard genetic techniques (Methods in Yeast Genetics, 2005, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 201-202) and transformants were selected on YEP medium supplemented 2% glucose and 100 μg/ml Geneticin at 30° C. Transformants were screened by colony PCR with primers LA468 (SEQ ID NO:161) and LA492 (SEQ ID NO:104) to verify presence of the integration cassette. A heterozygous diploid was obtained: NYLA98, which has the genotype MATa/α URA3/ura3::loxP-kanMX4-loxP. To obtain haploids, NYLA98 was sporulated using standard methods (Codón A C, Gasent-Ramirez J M, Benítez T. Factors which affect the frequency of sporulation and tetrad formation in *Saccharomyces cerevisiae* baker's yeast. Appl Environ Microbiol. 1995 PMID: 7574601). Tetrads were dissected using a micromanipulator and grown on rich YPE medium supplemented with 2% glucose. Tetrads containing four viable spores were patched onto synthetic complete medium lacking uracil supplemented with 2% glucose, and the mating type was verified by multiplex colony PCR using primers AK109-1 (SEQ ID NO:105), AK109-2 (SEQ ID NO: 106), and AK109-3 (SEQ ID NO:107). The resulting identified haploid strain called NYLA103, which has the genotype: MATα ura3Δ::loxP-kanMX4-loxP, and NYLA106, which has the genotype: MATa ura3Δ::loxP-kanMX4-loxP.

Deletion of His3

To delete the endogenous HIS3 coding region, a scarless deletion cassette was used. The four fragments for the PCR cassette for the scarless HIS3 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen; Valencia, Calif.). HIS3 Fragment A was amplified with primer oBP452 (SEQ ID NO:89) and primer oBP453 (SEQ ID NO:109), containing a 5' tail with homology to the 5' end of HIS3 Fragment B. HIS3 Fragment B was amplified with primer oBP454 (SEQ ID NO:110), containing a 5' tail with homology to the 3' end of HIS3 Fragment A, and primer oBP455 (SEQ ID NO:90) containing a 5' tail with homology to the 5' end of HIS3 Fragment U. HIS3 Fragment U was amplified with primer oBP456 (SEQ ID NO:91), containing a 5' tail with homology to the 3' end of HIS3 Fragment B, and primer oBP457 (SEQ ID NO:86), containing a 5' tail with homology to the 5' end of HIS3 Fragment C. HIS3 Fragment C was amplified with primer oBP458 (SEQ ID NO:87), containing a 5' tail with homology to the 3' end of HIS3 Fragment U, and primer oBP459 (SEQ ID NO:88). PCR products were purified with a PCR Purification kit (Qiagen). HIS3 Fragment AB was created by overlapping PCR by mixing HIS3 Fragment A and HIS3 Fragment B and amplifying with primers oBP452 (SEQ ID NO:89) and oBP455 (SEQ ID NO:90). HIS3 Fragment UC was created by overlapping PCR by mixing HIS3 Fragment U and HIS3 Fragment C and amplifying with primers oBP456 (SEQ ID NO:91) and oBP459 (SEQ ID NO:88). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The HIS3 ABUC cassette was created by overlapping PCR by mixing HIS3 Fragment AB and HIS3 Fragment UC and amplifying with primers oBP452 (SEQ ID NO:89) and oBP459 (SEQ ID NO:88). The PCR product was purified with a PCR Purification kit (Qiagen). Competent cells of NYLA106 were transformed with the HIS3 ABUC PCR cassette and were plated on synthetic complete medium lacking uracil supplemented with 2% glucose at 30° C. Transformants were screened to verify correct integration by replica plating onto synthetic complete medium lacking histidine and supplemented with 2% glucose at 30° C. Genomic DNA preps were made to verify the integration by PCR using primers oBP460 (SEQ ID NO:93) and LA135 (SEQ ID NO:94) for the 5' end and primers oBP461 (SEQ ID NO:95) and LA92 (SEQ ID NO:96) for the 3' end. The URA3 marker was recycled by plating on synthetic complete medium supplemented with 2% glucose and 5-FOA at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto SD −URA medium to verify the absence of growth. The resulting identified strain, called PNY2003 has the genotype: MATa ura3Δ::loxP-kanMX4-loxP his3Δ.

Deletion of PDC1

To delete the endogenous PDC1 coding region, a deletion cassette was PCR-amplified from pLA59 (SEQ ID NO:97), which contains a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and primers LA678 (SEQ ID NO:98) and LA679 (SEQ ID NO:99). The PDC1 portion of each primer was derived from the 5' region 50 bp downstream of the PDC1 start codon and 3' region 50 bp upstream of the stop codon such that integration of the URA3 cassette results in replacement of the PDC1 coding region but leaves the first 50 bp and the last 50 bp of the coding region. The PCR product was transformed into PNY2003 using standard genetic techniques and transformants were selected on synthetic complete medium lacking uracil and supplemented with 2% glucose at 30° C. Transformants were screened to verify correct integration by colony PCR using primers LA337 (SEQ ID NO:111), external to the 5' coding region and LA135 (SEQ ID NO:94), an internal primer to URA3.

Positive transformants were then screened by colony PCR using primers LA692 (SEQ ID NO: 112) and LA693 (SEQ ID NO:113), internal to the PDC1 coding region. The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete medium lacking histidine and supplemented with 2% glucose at 30° C. Transformants were plated on rich medium supplemented with 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete medium lacking uracil and supplemented with 2% glucose to verify absence of growth. The resulting identified strain, called PNY2008 has the genotype: MATa ura3Δ::loxP-kanMX4-loxP his3Δ pdc1Δ::loxP71/66.

Deletion of PDC5

To delete the endogenous PDC5 coding region, a deletion cassette was PCR-amplified from pLA59 (SEQ ID NO:97), which contains a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and primers LA722 (SEQ ID NO:185) and LA733 (SEQ ID NO:186). The PDC5 portion of each primer was derived from the 5' region 50 bp upstream of the PDC5 start codon and 3' region 50 bp downstream of the stop codon such that integration of the URA3 cassette results in replacement of the entire PDC5 coding region. The PCR product was transformed into PNY2008 using standard genetic techniques and transformants were selected on synthetic complete medium lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by colony PCR using primers LA453 (SEQ ID NO:187), external to the 5' coding region and LA135 (SEQ ID NO:94), an internal primer to URA3. Positive transformants were then screened by colony PCR using primers LA694 (SEQ ID NO:188) and LA695 (SEQ ID NO:189), internal to the PDC5 coding region. The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete medium lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich YEP medium supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete medium lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain, called PNY2009 has the genotype: MATa ura3Δ::loxP-kanMX4-loxP his3Δ pdc1Δ::loxP71/66 pdc5Δ::loxP71/66.

Deletion of FRA2

The FRA2 deletion was designed to delete 250 nucleotides from the 3' end of the coding sequence, leaving the first 113 nucleotides of the FRA2 coding sequence intact. An in-frame stop codon was present 7 nucleotides downstream of the deletion. The four fragments for the PCR cassette for the scarless FRA2 deletion were amplified using Phusion High Fidelity PCR Master Mix (New England BioLabs; Ipswich, Mass.) and CEN.PK 113-7D genomic DNA as template, prepared with a Gentra Puregene Yeast/Bact kit (Qiagen; Valencia, Calif.). FRA2 Fragment A was amplified with primer oBP594 (SEQ ID NO:190) and primer oBP595 (SEQ ID NO:191), containing a 5' tail with homology to the 5' end of FRA2 Fragment B. FRA2 Fragment B was amplified with primer oBP596 (SEQ ID NO:192), containing a 5' tail with homology to the 3' end of FRA2 Fragment A, and primer oBP597 (SEQ ID NO:193), containing a 5' tail with homology to the 5' end of FRA2 Fragment U. FRA2 Fragment U was amplified with primer oBP598 (SEQ ID NO:194), containing a 5' tail with homology to the 3' end of FRA2 Fragment B, and primer oBP599 (SEQ ID NO:195), containing a 5' tail with homology to the 5' end of FRA2 Fragment C. FRA2 Fragment C was amplified with primer oBP600 (SEQ ID NO:196), containing a 5' tail with homology to the 3' end of FRA2 Fragment U, and primer oBP601 (SEQ ID NO:197). PCR products were purified with a PCR Purification kit (Qiagen). FRA2 Fragment AB was created by overlapping PCR by mixing FRA2 Fragment A and FRA2 Fragment B and amplifying with primers oBP594 (SEQ ID NO:190) and oBP597 (SEQ ID NO:193). FRA2 Fragment UC was created by overlapping PCR by mixing FRA2 Fragment U and FRA2 Fragment C and amplifying with primers oBP598 (SEQ ID NO:194) and oBP601 (SEQ ID NO:197). The resulting PCR products were purified on an agarose gel followed by a Gel Extraction kit (Qiagen). The FRA2 ABUC cassette was created by overlapping PCR by mixing FRA2 Fragment AB and FRA2 Fragment UC and amplifying with primers oBP594 (SEQ ID NO:190) and oBP601 (SEQ ID NO:197). The PCR product was purified with a PCR Purification kit (Qiagen).

To delete the endogenous FRA2 coding region, the scarless deletion cassette obtained above was transformed into PNY2009 using standard techniques and plated on synthetic complete medium lacking uracil and supplemented with 1% ethanol. Genomic DNA preps were made to verify the integration by PCR using primers oBP602 (SEQ ID NO:198) and LA135 (SEQ ID NO:94) for the 5' end, and primers oBP602 (SEQ ID NO:198) and oBP603 (SEQ ID NO:199) to amplify the whole locus. The URA3 marker was recycled by plating on synthetic complete medium supplemented with 1% ethanol and 5-FOA (5-Fluoroorotic Acid) at 30° C. following standard protocols. Marker removal was confirmed by patching colonies from the 5-FOA plates onto synthetic complete medium lacking uracil and supplemented with 1% ethanol to verify the absence of growth. The resulting identified strain, PNY2037, has the genotype: MATa ura3Δ::loxP-kanMX4-loxP his3Δ pdc1Δ::loxP71/66 pdc5Δ::loxP71/66 fra2Δ.

Addition of Native 2 Micron Plasmid

The loxP71-URA3-loxP66 marker was PCR-amplified using Phusion DNA polymerase (New England BioLabs; Ipswich, Mass.) from pLA59 (SEQ ID NO:97), and transformed along with the LA811×LA817 (SEQ ID NOs:200, 201) and LA812×LA818 (SEQ ID NOs:202, 203) 2-micron plasmid fragments (amplified from the native 2-micron plasmid from CEN.PK 113-7D; Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre) into strain PNY2037 on SE −URA plates at 30° C. The resulting strain PNY2037 2μ::loxP71-URA3-loxP66 was transformed with pLA34 (pRS423::cre) (also called, pLA34) (SEQ ID NO:184) and selected on SE −HIS −URA plates at 30° C. Transformants were patched onto YP-1% galactose plates and allowed to grow for 48 hrs at 30° C. to induce Cre recombinase expression. Individual colonies were then patched onto SE −URA, SE −HIS, and YPE plates to confirm URA3 marker removal. The resulting identified strain, PNY2050, has the genotype: MATa ura3Δ::loxP-kanMX4-loxP, his3Δ pdc1Δ::loxP71/66 pdc5Δ::loxP71/66 fra2Δ 2-micron.

Construction of PNY2115 from PNY2050

Construction of PNY2115 [MATa ura3Δ::loxP his3Δ pdc5Δ::loxP66/71 fra2Δ 2-micron plasmid (CEN.PK2) pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66 adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66 fra2Δ::P

[ILV5]-ADH|Bi(y)-ADHt-loxP71/66 gpd2Δ::loxP71/66] from PNY2050 was as follows.

Pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66

To integrate alsS into the pdc1Δ::loxP66/71 locus of PNY2050 using the endogenous PDC1 promoter, an integration cassette was PCR-amplified from pLA71 (SEQ ID NO:209), which contains the gene acetolactate synthase from the species *Bacillus subtilis* with a FBA1 promoter and a CYC1 terminator, and a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using KAPA HiFi and primers 895 (SEQ ID NO:212) and 679 (SEQ ID NO:213). The PDC1 portion of each primer was derived from 60 bp of the upstream of the coding sequence and 50 bp that are 53 bp upstream of the stop codon. The PCR product was transformed into PNY2050 using standard genetic techniques and transformants were selected on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by colony PCR using primers 681 (SEQ ID NO:214), external to the 3' coding region and 92 (SEQ ID NO:215), internal to the URA3 gene. Positive transformants were then prepped for genomic DNA and screened by PCR using primers N245 (SEQ ID NO:216) and N246 (SEQ ID NO:217). The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete media lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich media supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete media lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain, called PNY2090 has the genotype MATa ura3Δ::loxP, his3Δ, pdc1Δ::loxP71/66, pdc5Δ::loxP71/66 fra2Δ 2-micron pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66.

Pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66

To delete the endogenous PDC6 coding region, an integration cassette was PCR-amplified from pLA78 (SEQ ID NO:210), which contains the kivD gene from the species *Listeria grayi* with a hybrid FBA1 promoter and a TDH3 terminator, and a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using KAPA HiFi and primers 896 (SEQ ID NO:218) and 897 (SEQ ID NO:219). The PDC6 portion of each primer was derived from 60 bp upstream of the coding sequence and 59 bp downstream of the coding region. The PCR product was transformed into PNY2090 using standard genetic techniques and transformants were selected on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by colony PCR using primers 365 (SEQ ID NO:220) and 366 (SEQ ID NO:221), internal primers to the PDC6 gene. Transformants with an absence of product were then screened by colony PCR N638 (SEQ ID NO:222), external to the 5' end of the gene, and 740 (SEQ ID NO:223), internal to the FBA1 promoter. Positive transformants were than the prepped for genomic DNA and screened by PCR with two external primers to the PDC6 coding sequence. Positive integrants would yield a 4720 bp product, while PDC6 wild type transformants would yield a 2130 bp product. The URA3 marker was recycled by transforming with pLA34 containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete media lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich media supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete media lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain is called PNY2093 and has the genotype MATa ura3Δ::loxP his3Δ pdc5Δ::loxP71/66 fra2Δ 2-micron pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66.

Adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66

To delete the endogenous ADH1 coding region and integrate BiADH using the endogenous ADH1 promoter, an integration cassette was PCR-amplified from pLA65 (SEQ ID NO:211), which contains the alcohol dehydrogenase from the species *Beijerinckii incida* with an ILV5 promoter and a ADH1 terminator, and a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using KAPA HiFi and primers 856 (SEQ ID NO:224) and 857 (SEQ ID NO:225). The ADH1 portion of each primer was derived from the 5' region 50 bp upstream of the ADH1 start codon and the last 50 bp of the coding region. The PCR product was transformed into PNY2093 using standard genetic techniques and transformants were selected on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by colony PCR using primers BK415 (SEQ ID NO:226), external to the 5' coding region and N1092 (SEQ ID NO:227), internal to the BiADH gene. Positive transformants were then screened by colony PCR using primers 413 (SEQ ID NO:160), external to the 3' coding region, and 92 (SEQ ID NO:215), internal to the URA3 marker. The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete media lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich media supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete media lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain, called PNY2101 has the genotype MATa ura3Δ::loxP his3Δ pdc5Δ::loxP71/66 fra2Δ 2-micron pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66 adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66.

Fra2Δ::P[ILV5]-ADH|Bi(y)-ADHt-loxP71/66

To integrate BiADH into the flan locus of PNY2101, an integration cassette was PCR-amplified from pLA65 (SEQ ID NO:211), which contains the alcohol dehydrogenase from the species *Beijerinckii indica* with an ILV5 promoter and an ADH1 terminator, and a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was performed by using KAPA HiFi and primers 906 (SEQ ID NO:228) and 907 (SEQ ID NO:229). The FRA2 portion of each primer was derived from the first 60 bp of the coding sequence starting at the ATG and 56 bp downstream of the stop codon. The PCR product was transformed into PNY2101 using standard genetic techniques and transformants were selected on synthetic complete media lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by

colony PCR using primers 667 (SEQ ID NO:230), external to the 5' coding region and 749 (SEQ ID NO:159), internal to the ILV5 promoter. The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete media lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich media supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete media lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain, called PNY2110 has the genotype MATa ura3Δ::loxP his3Δ pdc5Δ::loxP66/71 2-micron pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ:(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66 adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66 fra2Δ::P[ILV5]-ADH|Bi(y)-ADHt-loxP71/66.

GPD2 Deletion

To delete the endogenous GPD2 coding region, a deletion cassette was PCR amplified from pLA59 (SEQ ID NO:97), which contains a URA3 marker flanked by degenerate loxP sites to allow homologous recombination in vivo and subsequent removal of the URA3 marker. PCR was done by using KAPA HiFi and primers LA512 (SEQ ID NO:204) and LA513 (SEQ ID NO:205). The GPD2 portion of each primer was derived from the 5' region 50 bp upstream of the GPD2 start codon and 3' region 50 bp downstream of the stop codon such that integration of the URA3 cassette results in replacement of the entire GPD2 coding region. The PCR product was transformed into PNY2110 using standard genetic techniques and transformants were selected on synthetic complete medium lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were screened to verify correct integration by colony PCR using primers LA516 (SEQ ID NO:206) external to the 5' coding region and LA135 (SEQ ID NO:94), internal to URA3. Positive transformants were then screened by colony PCR using primers LA514 (SEQ ID NO:207) and LA515 (SEQ ID NO:208), internal to the GPD2 coding region. The URA3 marker was recycled by transforming with pLA34 (SEQ ID NO:184) containing the CRE recombinase under the GAL1 promoter and plated on synthetic complete medium lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were plated on rich medium supplemented with 1% ethanol and 0.5% galactose to induce the recombinase. Marker removal was confirmed by patching colonies to synthetic complete medium lacking uracil and supplemented with 1% ethanol to verify absence of growth. The resulting identified strain, called PNY2115, has the genotype MATa ura3Δ::loxP his3Δ pdc5Δ::loxP66/71 fra2Δ 2-micron pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66 adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66 fra2Δ::P[ILV5]-ADH|Bi(y)-ADHt-loxP71/66 gpd2Δ::loxP71/66.

Creation of PNY2145 from PNY2115

PNY2145 was constructed from PNY2115 by the additional integration of a phosphoketolase gene cassette at the pdc5Δ locus and by replacing the native AMN1 gene with a codon optimized verison of the ortholog from CEN.PK. Integration constructs are further described below.

pdc5Δ::FBA(L8)-xpk1-CYC1t-loxP71/66

The TEF(M4)-xpk1-CYC1t gene from pRS423::TEF(M4)-xpk1+ENO1-eutD (SEQ ID NO:162) was PCR amplified using primers N1341 and N1338 (SEQ ID NOs:163 and 164), generating a 3.1 kb product. The loxP-flanked URA3 gene cassette from pLA59 (SEQ ID NO:97) was amplified with primers N1033c and N1342 (SEQ ID NOs:165 and 166), generating a 1.6 kb product. The xpk1 and URA3 PCR products were fused by combining them without primers for an additional 10 cycles of PCR using Phusion DNA polymerase. The resulting reaction mix was then used as a template for a PCR reaction with KAPA Hi Fi and primers N1342 and N1364 (SEQ ID NOs:166 and 167). A 4.2 kb PCR product was recovered by purification from an electrophoresis agarose gel (Zymo kit). FBA promoter variant L8 (SEQ ID NO:168) was amplified using primers N1366 and N1368 (SEQ ID NOs:169 and 170). The xpk1::URA3 PCR product was combined with the FBA promoter by additional rounds of PCR. The resulting product was phosphorylated with polynucleotide kinase and ligated into pBR322 that had been digested with EcoRV and treated with calf intestinal phosphatase. The ligation reaction was transformed into *E. coli* cells (Stb13 competent cells from Invitrogen). The integration cassette was confirmed by sequencing. To prepare DNA for integration, the plasmid was used as a template in a PCR reaction with Kapa HiFi and primers N1371 and N1372 (SEQ ID NOs:171 and 172). The PCR product was isolated by phenol-chloroform extraction and ethanol precipitation (using standard methods; e.g. Maniatas, et al.). Five micrograms of DNA were used to transform strain PNY2115. Transformants were selected on medium lacking uracil (synthetic complete medium minus uracil with 1% ethanol as the carbon source). Colonies were screened for the integration event using PCR (JumpStart) with primers BK93 and N1114 (SEQ ID NOs:173 and 174). Two clones were selected to carry forward. The URA3 marker was recycled by transforming with pJT254 (SEQ ID NO:175) containing the CRE recombinase under the GAL1 promoter and plating on synthetic complete medium lacking histidine and supplemented with 1% ethanol at 30° C. Transformants were grown in rich medium supplemented with 1% ethanol to derepress the recombinase. Marker removal was confirmed for single colony isolates by patching to synthetic complete medium lacking uracil and supplemented with 1% ethanol to verify absence of growth. Loss of the recombinase plasmid, pJT254, was confirmed by patching the colonies to synthetic complete medium lacking histidine and supplemented with 1% ethanol. Proper marker removal was confirmed by PCR (primers N160SeqF5 (SEQ ID NO:176) and BK380. One resulting clone was designated PNY2293.

amn1Δ::AMN1(y)-loxP71/66

To replace the endogenous copy of AMN1 with a codon-optimized version of the AMN1 gene from CEN.PK2, an integration cassette containing the CEN.PK AMN1 promoter, AMN1(y) gene (nucleic acid SEQ ID NO:177; amino acid SEQ ID NO:178), and CEN.PK AMN1 terminator was assembled by SOE PCR and subcloned into the shuttle vector pLA59. The AMN1(y) gene was ordered from DNA 2.0 with codon-optimization for *S. cerevisiae*. The completed pLA67 plasmid (SEQ ID NO:179) contained: 1) pUC19 vector backbone sequence containing an *E. coli* replication origin and ampicillin resistance gene; 2) URA3 selection marker flanked by loxP71 and loxP66 sites; and 3) $P_{AMN1(CEN.PK)}$-AMN1(y)-$term_{AMN1(CEN.PK)}$ expression cassette

PCR amplification of the AMN1(y)-loxP71-URA3-loxP66 cassette was performed by using KAPA HiFi from

Kapa Biosystems, Woburn, Mass. and primers LA712 (SEQ ID NO:180) and LA746 (SEQ ID NO:181). The PCR product was transformed into PNY2293 using standard genetic techniques and transformants were selected on synthetic complete medium lacking uracil and supplemented with 1% ethanol at 30° C. Transformants were observed under magnification for the absence of a clumping phenotype with respect to the control (PNY2293). The URA3 marker was recycled using the pJT254 Cre recombinase plasmid as described above. After marker recycle, clones were again observed under magnification to confirm absence of the clumping phenotype. A resulting identified strain, PNY2145, has the genotype: MATa ura3Δ::loxP his3Δ pdc5Δ::P[FBA(L8)]-XPK|xpk1_Lp-CYCt-loxP66/71 fra2Δ 2-micron plasmid (CEN.PK2) pdc1Δ::P[PDC1]-ALS|alsS_Bs-CYC1t-loxP71/66 pdc6Δ::(UAS)PGK1-P[FBA1]-KIVD|Lg(y)-TDH3t-loxP71/66 adh1Δ::P[ADH1]-ADH|Bi(y)-ADHt-loxP71/66 fra2Δ::P[ILV5]-ADH|Bi(y)-ADHt-loxP71/66 gpd2Δ::loxP71/66 amn1Δ::AMN1(y).

INCORPORATION BY REFERENCE

All documents cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued or foreign patents, or any other documents, are each entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited documents.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

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<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

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            20                  25                  30

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

Ala Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Ile
    50                  55                  60

Lys Gly Met Ser Cys Ile Ile Thr Thr Phe Gly Val Gly Glu Leu Ser
65                  70                  75                  80

Ala Leu Asn Gly Ile Ala Gly Ser Tyr Ala Glu His Val Gly Val Leu
                85                  90                  95

His Val Val Gly Val Pro Ser Ile Ser Ser Gln Ala Lys Gln Leu Leu
            100                 105                 110

Leu His His Thr Leu Gly Asn Gly Asp Phe Thr Val Phe His Arg Met
        115                 120                 125

Ser Ala Asn Ile Ser Glu Thr Thr Ala Met Ile Thr Asp Ile Ala Asn
    130                 135                 140

Ala Pro Ala Glu Ile Asp Arg Cys Ile Arg Thr Thr Tyr Thr Thr Gln
145                 150                 155                 160

Arg Pro Val Tyr Leu Gly Leu Pro Ala Asn Leu Val Asp Leu Asn Val
                165                 170                 175

Pro Ala Lys Leu Leu Glu Thr Pro Ile Asp Leu Ser Leu Lys Pro Asn
            180                 185                 190

Asp Ala Glu Ala Glu Ala Glu Val Val Arg Thr Val Val Glu Leu Ile
        195                 200                 205

Lys Asp Ala Lys Asn Pro Val Ile Leu Ala Asp Ala Cys Ala Ser Arg
    210                 215                 220

His Asp Val Lys Ala Glu Thr Lys Lys Leu Met Asp Leu Thr Gln Phe
225                 230                 235                 240
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Pro Val Tyr Val Thr Pro Met Gly Lys Gly Ala Ile Asp Glu Gln His
                245                 250                 255

Pro Arg Tyr Gly Gly Val Tyr Val Gly Thr Leu Ser Arg Pro Glu Val
            260                 265                 270

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

Leu Ser Asp Phe Asn Thr Gly Ser Phe Ser Tyr Ser Tyr Lys Thr Lys
    290                 295                 300

Asn Ile Val Glu Phe His Ser Asp His Ile Lys Ile Arg Asn Ala Thr
305                 310                 315                 320

Phe Pro Gly Val Gln Met Lys Phe Ala Leu Gln Lys Leu Leu Asp Ala
                325                 330                 335

Ile Pro Glu Val Val Lys Asp Tyr Lys Pro Val Ala Val Pro Ala Arg
            340                 345                 350

Val Pro Ile Thr Lys Ser Thr Pro Ala Asn Thr Pro Met Lys Gln Glu
        355                 360                 365

Trp Met Trp Asn His Leu Gly Asn Phe Leu Arg Glu Gly Asp Ile Val
    370                 375                 380

Ile Ala Glu Thr Gly Thr Ser Ala Phe Gly Ile Asn Gln Thr Thr Phe
385                 390                 395                 400

Pro Thr Asp Val Tyr Ala Ile Val Gln Val Leu Trp Gly Ser Ile Gly
                405                 410                 415

Phe Thr Val Gly Ala Leu Leu Gly Ala Thr Met Ala Ala Glu Glu Leu
            420                 425                 430

Asp Pro Lys Lys Arg Val Ile Leu Phe Ile Gly Asp Gly Ser Leu Gln
        435                 440                 445

Leu Thr Val Gln Glu Ile Ser Thr Met Ile Arg Trp Gly Leu Lys Pro
    450                 455                 460

Tyr Ile Phe Val Leu Asn Asn Asn Gly Tyr Thr Ile Glu Lys Leu Ile
465                 470                 475                 480

His Gly Pro His Ala Glu Tyr Asn Glu Ile Gln Gly Trp Asp His Leu
                485                 490                 495

Ala Leu Leu Pro Thr Phe Gly Ala Arg Asn Tyr Glu Thr His Arg Val
            500                 505                 510

Ala Thr Thr Gly Glu Trp Glu Lys Leu Thr Gln Asp Lys Asp Phe Gln
        515                 520                 525

Asp Asn Ser Lys Ile Arg Met Ile Glu Val Met Leu Pro Val Phe Asp
    530                 535                 540

Ala Pro Gln Asn Leu Val Lys Gln Ala Gln Leu Thr Ala Ala Thr Asn
545                 550                 555                 560

Ala Lys Gln


<210> SEQ ID NO 2
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<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 2

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Val Asn Cys Asn Thr Val Phe Gly Leu Pro Gly Asp Phe Asn Leu Ser
            20                  25                  30

Leu Leu Asp Lys Leu Tyr Glu Val Lys Gly Met Arg Trp Ala Gly Asn
        35                  40                  45
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Ala Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Ile
    50                  55                  60

Lys Gly Met Ser Cys Ile Ile Thr Thr Phe Gly Val Gly Glu Leu Ser
65                  70                  75                  80

Ala Leu Asn Gly Ile Ala Gly Ser Tyr Ala Glu His Val Gly Val Leu
                85                  90                  95

His Val Val Gly Val Pro Ser Ile Ser Ser Gln Ala Lys Gln Leu Leu
            100                 105                 110

Leu His His Thr Leu Gly Asn Gly Asp Phe Thr Val Phe His Arg Met
        115                 120                 125

Ser Ala Asn Ile Ser Glu Thr Thr Ala Met Ile Thr Asp Ile Ala Asn
    130                 135                 140

Ala Pro Ala Glu Ile Asp Arg Cys Ile Arg Thr Thr Tyr Thr Thr Gln
145                 150                 155                 160

Arg Pro Val Tyr Leu Gly Leu Pro Ala Asn Leu Val Asp Leu Asn Val
                165                 170                 175

Pro Ala Lys Leu Leu Glu Thr Pro Ile Asp Leu Ser Leu Lys Pro Asn
            180                 185                 190

Asp Ala Glu Ala Glu Ala Glu Val Val Arg Thr Val Val Glu Leu Ile
        195                 200                 205

Lys Asp Ala Lys Asn Pro Val Ile Leu Ala Asp Ala Cys Ala Ser Arg
    210                 215                 220

His Asp Val Lys Ala Glu Thr Lys Lys Leu Met Asp Leu Thr Gln Phe
225                 230                 235                 240

Pro Val Tyr Val Thr Pro Met Gly Lys Gly Ala Ile Asp Glu Gln His
                245                 250                 255

Pro Arg Tyr Gly Gly Val Tyr Val Gly Thr Leu Ser Arg Pro Glu Val
            260                 265                 270

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

Leu Ser Asp Phe Asn Thr Gly Ser Phe Ser Tyr Ser Tyr Lys Thr Lys
    290                 295                 300

Asn Ile Val Glu Phe His Ser Asp His Ile Lys Ile Arg Asn Ala Thr
305                 310                 315                 320

Phe Pro Gly Val Gln Met Lys Phe Ala Leu Gln Lys Leu Leu Asp Ala
                325                 330                 335

Ile Pro Glu Val Val Lys Asp Tyr Lys Pro Val Ala Val Pro Ala Arg
            340                 345                 350

Val Pro Ile Thr Lys Ser Thr Pro Ala Asn Thr Pro Met Lys Gln Glu
        355                 360                 365

Trp Met Trp Asn His Leu Gly Asn Phe Leu Arg Glu Gly Asp Ile Val
    370                 375                 380

Ile Ala Glu Thr Gly Thr Ser Ala Phe Gly Ile Asn Gln Thr Thr Phe
385                 390                 395                 400

Pro Thr Asp Val Tyr Ala Ile Val Gln Val Leu Trp Gly Ser Ile Gly
                405                 410                 415

Phe Thr Val Gly Ala Leu Leu Gly Ala Thr Met Ala Ala Glu Glu Leu
            420                 425                 430

Asp Pro Lys Lys Arg Val Ile Leu Phe Ile Gly Asp Gly Ser Leu Gln
        435                 440                 445

Leu Thr Val Gln Glu Ile Ser Thr Met Ile Arg Trp Gly Leu Lys Pro
    450                 455                 460
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Tyr Ile Phe Val Leu Asn Asn Asn Gly Tyr Thr Ile Glu Lys Leu Ile
465                 470                 475                 480

His Gly Pro His Ala Glu Tyr Asn Glu Ile Gln Gly Trp Asp His Leu
                485                 490                 495

Ala Leu Leu Pro Thr Phe Gly Ala Arg Asn Tyr Glu Thr His Arg Val
            500                 505                 510

Ala Thr Thr Gly Glu Trp Glu Lys Leu Thr Gln Asp Lys Asp Phe Gln
        515                 520                 525

Asp Asn Ser Lys Ile Arg Met Ile Glu Val Met Leu Pro Val Phe Asp
    530                 535                 540

Ala Pro Gln Asn Leu Val Lys Gln Ala Gln Leu Thr Ala Ala Thr Asn
545                 550                 555                 560

Ala Lys Gln


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

<400> SEQUENCE: 3

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

Leu Leu Asp Lys Ile Tyr Glu Val Asp Gly Leu Arg Trp Ala Gly Asn
        35                  40                  45

Ala Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Ile
    50                  55                  60

Lys Gly Leu Ser Val Leu Val Thr Thr Phe Gly Val Gly Glu Leu Ser
65                  70                  75                  80

Ala Leu Asn Gly Ile Ala Gly Ser Tyr Ala Glu His Val Gly Val Leu
                85                  90                  95

His Val Val Gly Val Pro Ser Ile Ser Ala Gln Ala Lys Gln Leu Leu
            100                 105                 110

Leu His His Thr Leu Gly Asn Gly Asp Phe Thr Val Phe His Arg Met
        115                 120                 125

Ser Ala Asn Ile Ser Glu Thr Thr Ser Met Ile Thr Asp Ile Ala Thr
    130                 135                 140

Ala Pro Ser Glu Ile Asp Arg Leu Ile Arg Thr Thr Phe Ile Thr Gln
145                 150                 155                 160

Arg Pro Ser Tyr Leu Gly Leu Pro Ala Asn Leu Val Asp Leu Lys Val
                165                 170                 175

Pro Gly Ser Leu Leu Glu Lys Pro Ile Asp Leu Ser Leu Lys Pro Asn
            180                 185                 190

Asp Pro Glu Ala Glu Lys Glu Val Ile Asp Thr Val Leu Glu Leu Ile
        195                 200                 205

Gln Asn Ser Lys Asn Pro Val Ile Leu Ser Asp Ala Cys Ala Ser Arg
    210                 215                 220

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

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

Pro Arg Tyr Gly Gly Val Tyr Val Gly Thr Leu Ser Lys Gln Asp Val
            260                 265                 270
```

```
Lys Gln Ala Val Glu Ser Ala Asp Leu Ile Leu Ser Val Gly Ala Leu
        275                 280                 285

Leu Ser Asp Phe Asn Thr Gly Ser Phe Ser Tyr Ser Tyr Lys Thr Lys
    290                 295                 300

Asn Val Val Glu Phe His Ser Asp Tyr Val Lys Val Lys Asn Ala Thr
305                 310                 315                 320

Phe Leu Gly Val Gln Met Lys Phe Ala Leu Gln Asn Leu Leu Lys Val
                325                 330                 335

Ile Pro Asp Val Val Lys Gly Tyr Lys Ser Val Pro Val Pro Thr Lys
            340                 345                 350

Thr Pro Ala Asn Lys Gly Val Pro Ala Ser Thr Pro Leu Lys Gln Glu
        355                 360                 365

Trp Leu Trp Asn Glu Leu Ser Lys Phe Leu Gln Glu Gly Asp Val Ile
    370                 375                 380

Ile Ser Glu Thr Gly Thr Ser Ala Phe Gly Ile Asn Gln Thr Ile Phe
385                 390                 395                 400

Pro Lys Asp Ala Tyr Gly Ile Ser Gln Val Leu Trp Gly Ser Ile Gly
                405                 410                 415

Phe Thr Thr Gly Ala Thr Leu Gly Ala Ala Phe Ala Ala Glu Glu Ile
            420                 425                 430

Asp Pro Asn Lys Arg Val Ile Leu Phe Ile Gly Asp Gly Ser Leu Gln
        435                 440                 445

Leu Thr Val Gln Glu Ile Ser Thr Met Ile Arg Trp Gly Leu Lys Pro
    450                 455                 460

Tyr Leu Phe Val Leu Asn Asn Asp Gly Tyr Thr Ile Glu Lys Leu Ile
465                 470                 475                 480

His Gly Pro His Ala Glu Tyr Asn Glu Ile Gln Thr Trp Asp His Leu
                485                 490                 495

Ala Leu Leu Pro Ala Phe Gly Ala Lys Lys Tyr Glu Asn His Lys Ile
            500                 505                 510

Ala Thr Thr Gly Glu Trp Asp Ala Leu Thr Thr Asp Ser Glu Phe Gln
        515                 520                 525

Lys Asn Ser Val Ile
    530
```

<210> SEQ ID NO 4
<211> LENGTH: 804
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 4

```
atgtcccaag gtagaaaagc tgcagaaaga ttggctaaga agactgtcct cattacaggt     60

gcatctgctg gtattggtaa ggcgaccgca ttagagtact tggaggcatc caatggtgat    120

atgaaactga tcttggctgc tagaagatta gaaaagctcg aggaattgaa gaagaccatt    180

gatcaagagt ttccaaacgc aaaagttcat gtggcccagc tggatatcac tcaagcagaa    240

aaaatcaagc ccttcattga aaacttgcca caagagttca aggatattga cattctggtg    300

aacaatgccg gaaaggctct tggcagtgac cgtgtgggcc agatcgcaac ggaggatatc    360

caggacgtgt ttgacaccaa cgtcacggct ttaatcaata tcacacaagc tgtactgccc    420

atattccaag ccaagaattc aggagatatt gtaaatttgg gttcaatcgc tggcagagac    480

gcatacccaa caggttctat ctattgtgcc tctaagtttg ccgtgggggc gttcactgat    540

agtttgagaa aggagctcat caacactaaa attagagtca ttctaattgc accagggcta    600
```

```
gtcgagactg aattttcact agttagatac agaggtaacg aggaacaagc caagaatgtt    660

tacaaggata ctaccccatt gatggctgat gacgtggctg atctgatcgt ctatgcaact    720

tccagaaaac aaaatactgt aattgcagac actttaatct ttccaacaaa ccaagcgtca    780

cctcatcata tcttccgtgg ataa                                           804


<210> SEQ ID NO 5
<211> LENGTH: 267
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 5

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

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

Tyr Leu Glu Ala Ser Asn Gly Asp Met Lys Leu Ile Leu Ala Ala Arg
        35                  40                  45

Arg Leu Glu Lys Leu Glu Glu Leu Lys Lys Thr Ile Asp Gln Glu Phe
    50                  55                  60

Pro Asn Ala Lys Val His Val Ala Gln Leu Asp Ile Thr Gln Ala Glu
65                  70                  75                  80

Lys Ile Lys Pro Phe Ile Glu Asn Leu Pro Gln Glu Phe Lys Asp Ile
                85                  90                  95

Asp Ile Leu Val Asn Asn Ala Gly Lys Ala Leu Gly Ser Asp Arg Val
            100                 105                 110

Gly Gln Ile Ala Thr Glu Asp Ile Gln Asp Val Phe Asp Thr Asn Val
        115                 120                 125

Thr Ala Leu Ile Asn Ile Thr Gln Ala Val Leu Pro Ile Phe Gln Ala
    130                 135                 140

Lys Asn Ser Gly Asp Ile Val Asn Leu Gly Ser Ile Ala Gly Arg Asp
145                 150                 155                 160

Ala Tyr Pro Thr Gly Ser Ile Tyr Cys Ala Ser Lys Phe Ala Val Gly
                165                 170                 175

Ala Phe Thr Asp Ser Leu Arg Lys Glu Leu Ile Asn Thr Lys Ile Arg
            180                 185                 190

Val Ile Leu Ile Ala Pro Gly Leu Val Glu Thr Glu Phe Ser Leu Val
        195                 200                 205

Arg Tyr Arg Gly Asn Glu Glu Gln Ala Lys Asn Val Tyr Lys Asp Thr
    210                 215                 220

Thr Pro Leu Met Ala Asp Asp Val Ala Asp Leu Ile Val Tyr Ala Thr
225                 230                 235                 240

Ser Arg Lys Gln Asn Thr Val Ile Ala Asp Thr Leu Ile Phe Pro Thr
                245                 250                 255

Asn Gln Ala Ser Pro His His Ile Phe Arg Gly
            260                 265


<210> SEQ ID NO 6
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 6

Met Ser Ala Ala Thr Val Gly Lys Pro Ile Lys Cys Ile Ala Ala Val
1               5                   10                  15

Ala Tyr Asp Ala Lys Lys Pro Leu Ser Val Glu Glu Ile Thr Val Asp
```

```
            20                  25                  30

Ala Pro Lys Ala His Glu Val Arg Ile Lys Ile Glu Tyr Thr Ala Val
        35                  40                  45

Cys His Thr Asp Ala Tyr Thr Leu Ser Gly Ser Asp Pro Glu Gly Leu
    50                  55                  60

Phe Pro Cys Val Leu Gly His Glu Gly Ala Gly Ile Val Glu Ser Val
65                  70                  75                  80

Gly Asp Asp Val Ile Thr Val Lys Pro Gly Asp His Val Ile Ala Leu
                85                  90                  95

Tyr Thr Ala Glu Cys Gly Lys Cys Lys Phe Cys Thr Ser Gly Lys Thr
            100                 105                 110

Asn Leu Cys Gly Ala Val Arg Ala Thr Gln Gly Lys Gly Val Met Pro
        115                 120                 125

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

Phe Met Gly Cys Ser Thr Phe Ser Glu Tyr Thr Val Val Ala Asp Val
145                 150                 155                 160

Ser Val Val Ala Ile Asp Pro Lys Ala Pro Leu Asp Ala Ala Cys Leu
                165                 170                 175

Leu Gly Cys Gly Val Thr Thr Gly Phe Gly Ala Ala Leu Lys Thr Ala
            180                 185                 190

Asn Val Gln Lys Gly Asp Thr Val Ala Val Phe Gly Cys Gly Thr Val
        195                 200                 205

Gly Leu Ser Val Ile Gln Gly Ala Lys Leu Arg Gly Ala Ser Lys Ile
    210                 215                 220

Ile Ala Ile Asp Ile Asn Asn Lys Lys Lys Gln Tyr Cys Ser Gln Phe
225                 230                 235                 240

Gly Ala Thr Asp Phe Val Asn Pro Lys Glu Asp Leu Ala Lys Asp Gln
                245                 250                 255

Thr Ile Val Glu Lys Leu Ile Glu Met Thr Asp Gly Gly Leu Asp Phe
            260                 265                 270

Thr Phe Asp Cys Thr Gly Asn Thr Lys Ile Met Arg Asp Ala Leu Glu
        275                 280                 285

Ala Cys His Lys Gly Trp Gly Gln Ser Ile Ile Ile Gly Val Ala Ala
    290                 295                 300

Ala Gly Glu Glu Ile Ser Thr Arg Pro Phe Gln Leu Val Thr Gly Arg
305                 310                 315                 320

Val Trp Lys Gly Ser Ala Phe Gly Gly Ile Lys Gly Arg Ser Glu Met
                325                 330                 335

Gly Gly Leu Ile Lys Asp Tyr Gln Lys Gly Ala Leu Lys Val Glu Glu
            340                 345                 350

Phe Ile Thr His Arg Arg Pro Phe Lys Glu Ile Asn Gln Ala Phe Glu
        355                 360                 365

Asp Leu His Asn Gly Asp Cys Leu Arg Thr Val Leu Lys Ser Asp Glu
    370                 375                 380

Ile Lys
385


<210> SEQ ID NO 7
<211> LENGTH: 399
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida

<400> SEQUENCE: 7
```

```
Met Ser Gly Asn Arg Gly Val Val Tyr Leu Gly Ser Gly Lys Val Glu
1               5                   10                  15

Val Gln Lys Ile Asp Tyr Pro Lys Met Gln Asp Pro Arg Gly Lys Lys
            20                  25                  30

Ile Glu His Gly Val Ile Leu Lys Val Val Ser Thr Asn Ile Cys Gly
        35                  40                  45

Ser Asp Gln His Met Val Arg Gly Arg Thr Thr Ala Gln Val Gly Leu
    50                  55                  60

Val Leu Gly His Glu Ile Thr Gly Glu Val Ile Glu Lys Gly Arg Asp
65                  70                  75                  80

Val Glu Asn Leu Gln Ile Gly Asp Leu Val Ser Val Pro Phe Asn Val
                85                  90                  95

Ala Cys Gly Arg Cys Arg Ser Cys Lys Glu Met His Thr Gly Val Cys
            100                 105                 110

Leu Thr Val Asn Pro Ala Arg Ala Gly Gly Ala Tyr Gly Tyr Val Asp
        115                 120                 125

Met Gly Asp Trp Thr Gly Gly Gln Ala Glu Tyr Leu Leu Val Pro Tyr
    130                 135                 140

Ala Asp Phe Asn Leu Leu Lys Leu Pro Asp Arg Asp Lys Ala Met Glu
145                 150                 155                 160

Lys Ile Arg Asp Leu Thr Cys Leu Ser Asp Ile Leu Pro Thr Gly Tyr
                165                 170                 175

His Gly Ala Val Thr Ala Gly Val Gly Pro Gly Ser Thr Val Tyr Val
            180                 185                 190

Ala Gly Ala Gly Pro Val Gly Leu Ala Ala Ala Ala Ser Ala Arg Leu
        195                 200                 205

Leu Gly Ala Ala Val Val Ile Val Gly Asp Leu Asn Pro Ala Arg Leu
    210                 215                 220

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

Thr Pro Leu His Glu Gln Ile Ala Ala Leu Leu Gly Glu Pro Glu Val
                245                 250                 255

Asp Cys Ala Val Asp Ala Val Gly Phe Glu Ala Arg Gly His Gly His
            260                 265                 270

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

Gln Val Thr Arg Val Ala Gly Lys Ile Gly Ile Pro Gly Leu Tyr Val
    290                 295                 300

Thr Glu Asp Pro Gly Ala Val Asp Ala Ala Ala Lys Ile Gly Ser Leu
305                 310                 315                 320

Ser Ile Arg Phe Gly Leu Gly Trp Ala Lys Ser His Ser Phe His Thr
                325                 330                 335

Gly Gln Thr Pro Val Met Lys Tyr Asn Arg Ala Leu Met Gln Ala Ile
            340                 345                 350

Met Trp Asp Arg Ile Asn Ile Ala Glu Val Val Gly Val Gln Val Ile
        355                 360                 365

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

Pro Lys Lys Phe Val Ile Asp Pro His Lys Thr Phe Ser Ala Ala
385                 390                 395
```

<210> SEQ ID NO 8
<211> LENGTH: 2064
<212> TYPE: DNA

<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 8

```
atgatcagac aatctacgct aaaaaacttc gctattaagc gttgctttca acatatagca     60
taccgcaaca cacctgccat gagatcagta gctctcgcgc agcgctttta tagttcgtct    120
tcccgttatt acagtgcgtc tccattacca gcctctaaaa ggccagagcc tgctccaagt    180
ttcaatgttg atccattaga acagcccgct gaaccttcaa aattggctaa gaaactacgc    240
gctgagcctg acatggatac ctctttcgtc ggtttaactg gtggtcaaat atttaacgaa    300
atgatgtcca gacaaaacgt tgatactgta tttggttatc caggtggtgc tatcctacct    360
gtttacgatg ccattcataa cagtgataaa ttcaacttcg ttcttccaaa acacgaacaa    420
ggtgccggtc acatggcaga aggctacgcc agagcttctg gtaaaccagg tgttgtcttg    480
gttacttctg ggccaggtgc caccaatgtc gttactccaa tggcagatgc ctttgcagac    540
gggattccaa tggttgtctt tacagggcaa gtctcaacta gtgctatcgg tactgatgct    600
ttccaagagg ctgacgtcgt tggtatttct agatcttgta cgaaatggaa tgtcatggtc    660
aagtccgtgg aagaattgcc attgcgtatt aacgaggctt ttgaaattgc cacgagcggt    720
agaccgggac cagtcttggt cgatttacca aaggatgtta cagcagctat cttaagaaat    780
ccaattccaa caaaaacaac tcttccatca aacgcactaa accaattaac cagtcgcgca    840
caagatgaat ttgtcatgca aagtatcaat aaagcagcag atttgatcaa cttggcaaag    900
aaacctgtct tatacgtcgg tgctggtatt ttaaaccatg cagatggtcc aagattacta    960
aaagaattaa gtgaccgtgc tcaaatacct gtcaccacta ctttacaagg tttaggttca   1020
ttcgaccaag aagatccaaa atcattggat atgcttggta tgcacggttg tgctactgcc   1080
aacctggcag tgcaaaatgc cgacttgata attgcagttg gtgctagatt cgacgaccgt   1140
gtcactggta atatttctaa attcgctcca gaagctcgtc gtgcagctgc cgagggtaga   1200
ggtggtatta ttcatttcga ggttagtcca aaaaacataa acaaggttgt tcaaactcaa   1260
atagcagtgg aaggtgatgc tacgaccaat ctgggcaaaa tgatgtcaaa gattttccca   1320
gttaaggaga ggtctgaatg gtttgctcaa ataaataaat ggaagaagga atacccatac   1380
gcttatatgg aggagactcc aggatctaaa attaaaccac agacggttat aaagaaacta   1440
tccaaggttg ccaacgacac aggaagacat gtcattgtta caacgggtgt ggggcaacat   1500
caaatgtggg ctgctcaaca ctggacatgg agaaatccac atactttcat cacatcaggt   1560
ggtttaggta cgatgggtta cggtctccct gccgccatcg gtgctcaagt tgcaaagcca   1620
gaatctttgg ttattgacat tgatggtgac gcatccttta acatgactct aacggaattg   1680
agttctgccg ttcaagctgg tactccagtg aagattttga ttttgaacaa tgaagagcaa   1740
ggtatggtta ctcaatggca atccctgttc tacgaacatc gttattccca cacacatcaa   1800
ttgaaccctg atttcataaa actagcggag gctatgggtt taaaaggttt aagagtcaag   1860
aagcaagagg aattggacgc taagttgaaa gaattcgttt ctaccaaggg cccagttttg   1920
cttgaagtgg aagttgataa aaaagttcct gttttgccaa tggtggcagg tggtagcggt   1980
ctagacgagt tcataaattt tgacccagaa gttgaaagac aacagactga attacgtcat   2040
aagcgtacag gcggtaagca ctga                                          2064
```

<210> SEQ ID NO 9
<211> LENGTH: 687
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 9

```
Met Ile Arg Gln Ser Thr Leu Lys Asn Phe Ala Ile Lys Arg Cys Phe
1               5                   10                  15

Gln His Ile Ala Tyr Arg Asn Thr Pro Ala Met Arg Ser Val Ala Leu
            20                  25                  30

Ala Gln Arg Phe Tyr Ser Ser Ser Ser Arg Tyr Tyr Ser Ala Ser Pro
        35                  40                  45

Leu Pro Ala Ser Lys Arg Pro Glu Pro Ala Pro Ser Phe Asn Val Asp
    50                  55                  60

Pro Leu Glu Gln Pro Ala Glu Pro Ser Lys Leu Ala Lys Lys Leu Arg
65                  70                  75                  80

Ala Glu Pro Asp Met Asp Thr Ser Phe Val Gly Leu Thr Gly Gly Gln
                85                  90                  95

Ile Phe Asn Glu Met Met Ser Arg Gln Asn Val Asp Thr Val Phe Gly
            100                 105                 110

Tyr Pro Gly Gly Ala Ile Leu Pro Val Tyr Asp Ala Ile His Asn Ser
        115                 120                 125

Asp Lys Phe Asn Phe Val Leu Pro Lys His Glu Gln Gly Ala Gly His
    130                 135                 140

Met Ala Glu Gly Tyr Ala Arg Ala Ser Gly Lys Pro Gly Val Val Leu
145                 150                 155                 160

Val Thr Ser Gly Pro Gly Ala Thr Asn Val Val Thr Pro Met Ala Asp
                165                 170                 175

Ala Phe Ala Asp Gly Ile Pro Met Val Val Phe Thr Gly Gln Val Ser
            180                 185                 190

Thr Ser Ala Ile Gly Thr Asp Ala Phe Gln Glu Ala Asp Val Val Gly
        195                 200                 205

Ile Ser Arg Ser Cys Thr Lys Trp Asn Val Met Val Lys Ser Val Glu
    210                 215                 220

Glu Leu Pro Leu Arg Ile Asn Glu Ala Phe Glu Ile Ala Thr Ser Gly
225                 230                 235                 240

Arg Pro Gly Pro Val Leu Val Asp Leu Pro Lys Asp Val Thr Ala Ala
                245                 250                 255

Ile Leu Arg Asn Pro Ile Pro Thr Lys Thr Thr Leu Pro Ser Asn Ala
            260                 265                 270

Leu Asn Gln Leu Thr Ser Arg Ala Gln Asp Glu Phe Val Met Gln Ser
        275                 280                 285

Ile Asn Lys Ala Ala Asp Leu Ile Asn Leu Ala Lys Lys Pro Val Leu
    290                 295                 300

Tyr Val Gly Ala Gly Ile Leu Asn His Ala Asp Gly Pro Arg Leu Leu
305                 310                 315                 320

Lys Glu Leu Ser Asp Arg Ala Gln Ile Pro Val Thr Thr Thr Leu Gln
                325                 330                 335

Gly Leu Gly Ser Phe Asp Gln Glu Asp Pro Lys Ser Leu Asp Met Leu
            340                 345                 350

Gly Met His Gly Cys Ala Thr Ala Asn Leu Ala Val Gln Asn Ala Asp
        355                 360                 365

Leu Ile Ile Ala Val Gly Ala Arg Phe Asp Asp Arg Val Thr Gly Asn
    370                 375                 380

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

Gly Gly Ile Ile His Phe Glu Val Ser Pro Lys Asn Ile Asn Lys Val
```

```
                405                 410                 415

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

Lys Met Met Ser Lys Ile Phe Pro Val Lys Glu Arg Ser Glu Trp Phe
        435                 440                 445

Ala Gln Ile Asn Lys Trp Lys Lys Glu Tyr Pro Tyr Ala Tyr Met Glu
    450                 455                 460

Glu Thr Pro Gly Ser Lys Ile Lys Pro Gln Thr Val Ile Lys Lys Leu
465                 470                 475                 480

Ser Lys Val Ala Asn Asp Thr Gly Arg His Val Ile Val Thr Thr Gly
                485                 490                 495

Val Gly Gln His Gln Met Trp Ala Ala Gln His Trp Thr Trp Arg Asn
            500                 505                 510

Pro His Thr Phe Ile Thr Ser Gly Gly Leu Gly Thr Met Gly Tyr Gly
        515                 520                 525

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

Ile Asp Ile Asp Gly Asp Ala Ser Phe Asn Met Thr Leu Thr Glu Leu
545                 550                 555                 560

Ser Ser Ala Val Gln Ala Gly Thr Pro Val Lys Ile Leu Ile Leu Asn
                565                 570                 575

Asn Glu Glu Gln Gly Met Val Thr Gln Trp Gln Ser Leu Phe Tyr Glu
            580                 585                 590

His Arg Tyr Ser His Thr His Gln Leu Asn Pro Asp Phe Ile Lys Leu
        595                 600                 605

Ala Glu Ala Met Gly Leu Lys Gly Leu Arg Val Lys Lys Gln Glu Glu
    610                 615                 620

Leu Asp Ala Lys Leu Lys Glu Phe Val Ser Thr Lys Gly Pro Val Leu
625                 630                 635                 640

Leu Glu Val Glu Val Asp Lys Lys Val Pro Val Leu Pro Met Val Ala
                645                 650                 655

Gly Gly Ser Gly Leu Asp Glu Phe Ile Asn Phe Asp Pro Glu Val Glu
            660                 665                 670

Arg Gln Gln Thr Glu Leu Arg His Lys Arg Thr Gly Gly Lys His
        675                 680                 685


<210> SEQ ID NO 10
<211> LENGTH: 299
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 10

Met Thr Asp Ile Gly Arg Thr Lys Ser Arg Asn Tyr Lys Cys Ser Phe
1               5                   10                  15

Asp Gly Cys Glu Lys Val Tyr Asn Arg Pro Ser Leu Leu Gln Gln His
            20                  25                  30

Gln Asn Ser His Thr Asn Gln Lys Pro Tyr His Cys Asp Glu Pro Gly
        35                  40                  45

Cys Gly Lys Lys Phe Ile Arg Pro Cys His Leu Arg Val His Lys Trp
    50                  55                  60

Thr His Ser Gln Ile Lys Pro Lys Ala Cys Thr Leu Cys Gln Lys Arg
65                  70                  75                  80

Phe Val Thr Asn Gln Gln Leu Arg Arg His Leu Asn Ser His Glu Arg
                85                  90                  95
```

```
Lys Ser Lys Leu Ala Ser Arg Ile Asp Arg Lys His Glu Gly Val Asn
            100                 105                 110

Ala Asn Val Lys Ala Glu Leu Asn Gly Lys Glu Gly Gly Phe Asp Pro
        115                 120                 125

Lys Leu Pro Ser Gly Ser Pro Met Cys Gly Glu Glu Phe Ser Gln Gly
    130                 135                 140

His Leu Pro Gly Tyr Asp Asp Met Gln Val Leu Gln Cys Pro Tyr Lys
145                 150                 155                 160

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

Leu Gln His His Ile Ala Ser Lys Leu Val Val Pro Ser Gly Asp Pro
            180                 185                 190

Ser Leu Lys Glu Ser Leu Pro Thr Ser Glu Lys Ser Ser Ser Thr Asp
        195                 200                 205

Thr Thr Ser Ile Pro Gln Leu Ser Phe Ser Thr Thr Gly Thr Ser Ser
    210                 215                 220

Ser Glu Ser Val Asp Ser Thr Thr Ala Gln Thr Pro Thr Asp Pro Glu
225                 230                 235                 240

Ser Tyr Trp Ser Asp Asn Arg Cys Lys His Ser Asp Cys Gln Glu Leu
                245                 250                 255

Ser Pro Phe Ala Ser Val Phe Asp Leu Ile Asp His Tyr Asp His Thr
            260                 265                 270

His Ala Phe Ile Pro Glu Thr Leu Val Lys Tyr Ser Tyr Ile His Leu
        275                 280                 285

Tyr Lys Pro Ser Val Trp Asp Leu Phe Glu Tyr
    290                 295


<210> SEQ ID NO 11
<211> LENGTH: 299
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FZF1-4

<400> SEQUENCE: 11

Met Thr Asp Ile Gly Arg Thr Lys Ser Arg Asn Tyr Lys Cys Ser Phe
1               5                   10                  15

Asp Gly Cys Glu Lys Val Tyr Asn Arg Pro Ser Leu Leu Gln Gln His
            20                  25                  30

Gln Asn Ser His Thr Asn Gln Lys Pro Tyr His Cys Asp Glu Pro Gly
        35                  40                  45

Cys Gly Lys Lys Phe Ile Arg Pro Tyr His Leu Arg Val His Lys Trp
    50                  55                  60

Thr His Ser Gln Ile Lys Pro Lys Ala Cys Thr Leu Cys Gln Lys Arg
65                  70                  75                  80

Phe Val Thr Asn Gln Gln Leu Arg Arg His Leu Asn Ser His Glu Arg
                85                  90                  95

Lys Ser Lys Leu Ala Ser Arg Ile Asp Arg Lys His Glu Gly Val Asn
            100                 105                 110

Ala Asn Val Lys Ala Glu Leu Asn Gly Lys Glu Gly Gly Phe Asp Pro
        115                 120                 125

Lys Leu Pro Ser Gly Ser Pro Met Cys Gly Glu Glu Phe Ser Gln Gly
    130                 135                 140

His Leu Pro Gly Tyr Asp Asp Met Gln Val Leu Gln Cys Pro Tyr Lys
145                 150                 155                 160
```

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

Leu Gln His His Ile Ala Ser Lys Leu Val Val Pro Ser Gly Asp Pro
            180                 185                 190

Ser Leu Lys Glu Ser Leu Pro Thr Ser Glu Lys Ser Ser Ser Thr Asp
        195                 200                 205

Thr Thr Ser Ile Pro Gln Leu Ser Phe Ser Thr Thr Gly Thr Ser Ser
    210                 215                 220

Ser Glu Ser Val Asp Ser Thr Thr Ala Gln Thr Pro Thr Asp Pro Glu
225                 230                 235                 240

Ser Tyr Trp Ser Asp Asn Arg Cys Lys His Ser Asp Cys Gln Glu Leu
                245                 250                 255

Ser Pro Phe Ala Ser Val Phe Asp Leu Ile Asp His Tyr Asp His Thr
            260                 265                 270

His Ala Phe Ile Pro Glu Thr Leu Val Lys Tyr Ser Tyr Ile His Leu
        275                 280                 285

Tyr Lys Pro Ser Val Trp Asp Leu Phe Glu Tyr
    290                 295


<210> SEQ ID NO 12
<211> LENGTH: 458
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 12

Met Val Ala Asn Trp Val Leu Ala Leu Thr Arg Gln Phe Asp Pro Phe
1               5                   10                  15

Met Phe Met Met Val Met Gly Val Gly Ile Ser Ser Asn Ile Leu Tyr
            20                  25                  30

Ser Phe Pro Tyr Pro Ala Arg Trp Leu Arg Ile Cys Ser Tyr Ile Met
        35                  40                  45

Phe Ala Ile Ala Cys Leu Ile Phe Ile Ala Val Gln Ala Leu Gln Ile
    50                  55                  60

Leu His Leu Ile Val Tyr Ile Lys Glu Lys Ser Phe Arg Glu Tyr Phe
65                  70                  75                  80

Asn Asp Phe Phe Arg Asn Met Lys His Asn Leu Phe Trp Gly Thr Tyr
                85                  90                  95

Pro Met Gly Leu Val Thr Ile Ile Asn Phe Leu Gly Ala Leu Ser Lys
            100                 105                 110

Ala Asn Thr Thr Lys Ser Pro Thr Asn Ala Arg Asn Leu Met Ile Phe
        115                 120                 125

Val Tyr Val Leu Trp Trp Tyr Asp Leu Ala Val Cys Leu Val Ile Ala
    130                 135                 140

Trp Gly Ile Ser Phe Leu Ile Trp His Asp Tyr Tyr Pro Leu Glu Gly
145                 150                 155                 160

Ile Gly Asn Tyr Pro Ser Tyr Asn Ile Lys Met Ala Ser Glu Asn Met
                165                 170                 175

Lys Ser Val Leu Leu Leu Asp Ile Ile Pro Leu Val Val Val Ala Ser
            180                 185                 190

Ser Cys Gly Thr Phe Thr Met Ser Glu Ile Phe Phe His Ala Phe Asn
        195                 200                 205

Arg Asn Ile Gln Leu Ile Thr Leu Val Ile Cys Ala Leu Thr Trp Leu
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His Ala Ile Ile Phe Val Phe Ile Leu Ile Ala Ile Tyr Phe Trp Ser
225                 230                 235                 240
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Leu Tyr Ile Asn Lys Ile Pro Pro Met Thr Gln Val Phe Thr Leu Phe
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Leu Leu Leu Gly Pro Met Gly Gln Gly Ser Phe Gly Val Leu Leu Leu
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Thr Asp Asn Ile Lys Lys Tyr Ala Gly Lys Tyr Tyr Pro Thr Asp Asn
        275                 280                 285

Ile Thr Arg Glu Gln Glu Ile Leu Thr Ile Ala Val Pro Trp Cys Phe
    290                 295                 300

Lys Ile Leu Gly Met Val Ser Ala Met Ala Leu Leu Ala Met Gly Tyr
305                 310                 315                 320

Phe Phe Thr Val Ile Ser Val Val Ser Ile Leu Ser Tyr Tyr Asn Lys
                325                 330                 335

Lys Glu Ile Glu Asn Glu Thr Gly Lys Val Lys Arg Val Tyr Thr Phe
            340                 345                 350

His Lys Gly Phe Trp Gly Met Thr Phe Pro Met Gly Thr Met Ser Leu
        355                 360                 365

Gly Asn Glu Glu Leu Tyr Val Gln Tyr Asn Gln Tyr Val Pro Leu Tyr
    370                 375                 380

Ala Phe Arg Val Leu Gly Thr Ile Tyr Gly Gly Val Cys Val Cys Trp
385                 390                 395                 400

Ser Ile Leu Cys Leu Leu Cys Thr Leu His Glu Tyr Ser Lys Lys Met
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Leu His Ala Ala Arg Lys Ser Ser Leu Phe Ser Glu Ser Gly Thr Glu
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Lys Thr Thr Val Ser Pro Tyr Asn Ser Ile Glu Ser Val Glu Glu Ser
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Asn Ser Ala Leu Asp Phe Thr Arg Leu Ala
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<210> SEQ ID NO 13
<211> LENGTH: 427
<212> TYPE: PRT
<213> ORGANISM: Salmonella typhi

<400> SEQUENCE: 13

Met Glu Ser Leu Thr Leu Gln Pro Ile Ala Arg Val Asp Gly Ala Ile
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Asn Leu Pro Gly Ser Lys Ser Val Ser Asn Arg Ala Leu Leu Leu Ala
            20                  25                  30

Ala Leu Ala Cys Gly Lys Thr Val Leu Thr Asn Leu Leu Asp Ser Asp
        35                  40                  45

Asp Val Arg His Met Leu Asn Ala Leu Ser Ala Leu Gly Ile Asn Tyr
    50                  55                  60

Thr Leu Ser Ala Asp Arg Thr Arg Cys Asp Ile Thr Gly Asn Gly Gly
65                  70                  75                  80

Pro Leu Arg Ala Ser Gly Thr Leu Glu Leu Phe Leu Gly Asn Ala Gly
                85                  90                  95

Thr Ala Met Arg Pro Leu Ala Ala Ala Leu Cys Leu Gly Gln Asn Glu
            100                 105                 110

Ile Val Leu Thr Gly Glu Pro Arg Met Lys Glu Arg Pro Ile Gly His
        115                 120                 125

Leu Val Asp Ser Leu Arg Gln Gly Gly Ala Asn Ile Asp Tyr Leu Glu
    130                 135                 140

Gln Glu Asn Tyr Pro Pro Leu Arg Leu Arg Gly Gly Phe Ile Gly Gly
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145                 150                 155                 160

Asp Ile Glu Val Asp Gly Ser Val Ser Ser Gln Phe Leu Thr Ala Leu
                165                 170                 175

Leu Met Thr Ala Pro Leu Ala Pro Glu Asp Thr Ile Ile Arg Val Lys
            180                 185                 190

Gly Glu Leu Val Ser Lys Pro Tyr Ile Asp Ile Thr Leu Asn Leu Met
        195                 200                 205

Lys Thr Phe Gly Val Glu Ile Ala Asn His His Tyr Gln Gln Phe Val
    210                 215                 220

Val Lys Gly Gly Gln Gln Tyr His Ser Pro Gly Arg Tyr Leu Val Glu
225                 230                 235                 240

Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Ala Gly Ala Ile Lys
                245                 250                 255

Gly Gly Thr Val Lys Val Thr Gly Ile Gly Arg Lys Ser Met Gln Gly
            260                 265                 270

Asp Ile Arg Phe Ala Asp Val Leu Glu Lys Met Gly Ala Thr Ile Thr
        275                 280                 285

Trp Gly Asp Asp Phe Ile Ala Cys Thr Arg Gly Glu Leu His Ala Ile
    290                 295                 300

Asp Met Asp Met Asn His Ile Pro Asp Ala Ala Met Thr Ile Ala Thr
305                 310                 315                 320

Thr Ala Leu Phe Ala Lys Gly Thr Thr Thr Leu Arg Asn Ile Tyr Asn
                325                 330                 335

Trp Arg Val Lys Glu Thr Asp Arg Leu Phe Ala Met Ala Thr Glu Leu
            340                 345                 350

Arg Lys Val Gly Ala Glu Val Glu Glu Gly His Asp Tyr Ile Arg Ile
        355                 360                 365

Thr Pro Pro Ala Lys Leu Gln His Ala Asp Ile Gly Thr Tyr Asn Asp
    370                 375                 380

His Arg Met Ala Met Cys Phe Ser Leu Val Ala Leu Ser Asp Thr Pro
385                 390                 395                 400

Val Thr Ile Leu Asp Pro Lys Cys Thr Ala Lys Thr Phe Pro Asp Tyr
                405                 410                 415

Phe Glu Gln Leu Ala Arg Met Ser Thr Pro Ala
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<210> SEQ ID NO 14
<211> LENGTH: 9089
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: constructed plasmid

<400> SEQUENCE: 14

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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc    180

accataccac agcttttcaa ttcaattcat cattttttt ttattctttt ttttgatttc    240

ggtttctttg aaattttttt gattcggtaa tctccgaaca gaaggaagaa cgaaggaagg    300

agcacagact tagattggta tatatacgca tatgtagtgt tgaagaaaca tgaaattgcc    360

cagtattctt aacccaactg cacagaacaa aaacctgcag gaaacgaaga taaatcatgt    420

cgaaagctac atataaggaa cgtgctgcta ctcatcctag tcctgttgct gccaagctat    480
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<210> SEQ ID NO 15
<211> LENGTH: 5956
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pBTX1 plasmid

<400> SEQUENCE: 15

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acgacttatc gccactggca gcagccactg gtaacaggat tagcagagcg aggtatgtag   1920

gcggtgctac agagttcttg aagtggtggc ctaactacgg ctacactaga aggacagtat   1980

ttggtatctg cgctctgctg aagccagtta ccttcggaaa aagagttggt agctcttgat   2040

ccggcaaaca aaccaccgct ggtagcggtg gtttttttgt ttgcaagcag cagattacgc   2100

gcagaaaaaa aggatctcaa gaagatcctt tgatcttttc tacggggtct gacgctcagt   2160

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gactccaacg tcaaagggcg aaaaaccgtc tatcagggcg atggcccact acgtgaacca   5520

tcaccctaat caagtttttt ggggtcgagg tgccgtaaag cactaaatcg gaaccctaaa   5580

gggagccccc gatttagagc ttgacgggga aagccggcga acgtggcgag aaaggaaggg   5640

aagaaagcga aaggagcggg cgctagggcg ctggcaagtg tagcggtcac gctgcgcgta   5700

accaccacac ccgccgcgct taatgcgccg ctacagggcg cgtcgcgcca ttcgccattc   5760

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gcgaaagggg gatgtgctgc aaggcgatta agttgggtaa cgccagggtt ttcccagtca   5880

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<210> SEQ ID NO 16
<211> LENGTH: 570
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 16

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

Phe Gly Ile Pro Gly Ala Lys Ile Asp Ala Val Phe Asp Ala Leu Gln
        35                  40                  45

Asp Lys Gly Pro Glu Ile Ile Val Ala Arg His Glu Gln Asn Ala Ala
    50                  55                  60

Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly Val Val
65                  70                  75                  80

Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly Leu Leu
                85                  90                  95

Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly Asn Val
            100                 105                 110

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

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

Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala Ser Ala
145                 150                 155                 160

Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val Val Asn
                165                 170                 175

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

Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys Ile Gln
        195                 200                 205

Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly Arg Pro
    210                 215                 220

Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln Leu Pro
225                 230                 235                 240

Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp Leu Glu
                245                 250                 255
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Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro Gly Asp
            260                 265                 270

Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr Asp Pro
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Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg Thr Ile
    290                 295                 300

Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr Gln Pro
305                 310                 315                 320

Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His Ile Glu
                325                 330                 335

His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys Ile Leu
            340                 345                 350

Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro Ala Asp
        355                 360                 365

Trp Lys Ser Asp Arg Ala His Pro Leu Glu Ile Val Lys Glu Leu Arg
    370                 375                 380

Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly Ser His
385                 390                 395                 400

Ala Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu Thr Leu
                405                 410                 415

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

Ile Gly Ala Ser Leu Val Lys Pro Gly Glu Lys Val Val Ser Val Ser
        435                 440                 445

Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr Ala Val
    450                 455                 460

Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser Thr Tyr
465                 470                 475                 480

Asp Met Val Ala Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr Ser Ala
                485                 490                 495

Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser Phe Gly
            500                 505                 510

Ala Thr Gly Leu Arg Val Glu Ser Pro Asp Gln Leu Ala Asp Val Leu
        515                 520                 525

Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val Pro Val
    530                 535                 540

Asp Tyr Ser Asp Asn Ile Asn Leu Ala Ser Asp Lys Leu Pro Lys Glu
545                 550                 555                 560

Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
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<210> SEQ ID NO 17
<211> LENGTH: 4531
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pUC19 ILV2

<400> SEQUENCE: 17

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aatgtcatat ataagaagca cgattaaata ataataaagt ctgcattttt tactgaaaat     180

gcttttgaaa taaatgtttt tgaaattcag tgcttaccgc ctgtacgctt atgacgtaat     240
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tcagtctgtt gtctttcaac ttctgggtca aaatttatga actcgtctag accgctacca    300
cctgccacca ttggcaaaac aggaactttt ttatcaactt ccacttcaag caaaactggg    360
cccttggtag aaacgaattc tttcaactta gcgtccaatt cctcttgctt cttgactctt    420
aaacctttta aacccatagc ctccgctagt tttatgaaat cagggttcaa ttgatgtgtg    480
tgggaataac gatgttcgta gaacagggat tgccattgag taaccatacc ttgctcttca    540
ttgttcaaaa tcaaaatctt cactggagta ccagcttgaa cggcagaact caattccgtt    600
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gcagctgcac gacgagcttc tggagcgaat ttagaaatat taccagtgac acggtcgtcg   1140
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caaccgtgca taccaagcat atccaatgat tttggatctt cttggtcgaa tgaacctaaa   1260
ccttgtaaag tagtggtgac aggtatttga gcacggtcac ttaattcttt tagtaatctt   1320
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gccggaaggg ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt 3300

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tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa agggggatgt 4440

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acggccagtg aattcgagct cggtacccgg g 4531

<210> SEQ ID NO 18
<211> LENGTH: 6074
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLA59 ILV2-410

<400> SEQUENCE: 18

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cagaaggaag aacgaaggaa ggagcacaga cttagattgg tatatatacg catatgtagt    240
gttgaagaaa catgaaattg cccagtattc ttaacccaac tgcacagaac aaaaacctgc    300
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aagccgctaa aggcattatc cgccaagtac aatttttac tcttcgaaga cagaaaattt     600
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tgggcagaca ttacgaatgc acacggtgtg gtgggcccag gtattgttag cggtttgaag    720
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<212> TYPE: DNA
<213> ORGANISM: Lactococcus lactis

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Gly Leu Ile Gly Leu Gln Phe His Thr Ile Gly Val Ser Asp Gly Ile
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Ala Asn Gly Lys Leu Gly Met Arg Tyr Ser Leu Val Ser Arg Glu Val
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cacaagtttg tttgcttttc gtgcatgata ttaaatagct tggcagcaac aggactagga  12960
tgagtagcag cacgttcctt atatgtagct ttcgacatga tttatcttcg tttcctgcag  13020
gtttttgttc tgtgcagttg ggttaagaat actgggcaat ttcatgtttc ttcaacacta  13080
catatgcgta tatataccaa tctaagtctg tgctccttcc ttcgttcttc cttctgttcg  13140
gagattaccg aatcaaaaaa atttcaagga aaccgaaatc aaaaaaaaga ataaaaaaaa  13200
aatgatgaat tgaaaagctt gcatgcctgc aggtcgactc tagtatactc cgtctactgt  13260
acgatacact tccgctcagg tccttgtcct ttaacgaggc cttaccactc ttttgttact  13320
ctattgatcc agctcagcaa aggcagtgtg atctaagatt ctatcttcgc gatgtagtaa  13380
aactagctag accgagaaag agactagaaa tgcaaaaggc acttctacaa tggctgccat  13440
cattattatc cgatgtgacg ctgcattttt tttttttttt tttttttttt tttttttttt  13500
tttttttttt tttttttgta caaatatcat aaaaaaagag aatcttttta agcaaggatt  13560
```

```
ttcttaactt cttcggcgac agcatcaccg acttcggtgg tactgttgga accacctaaa  13620
tcaccagttc tgatacctgc atccaaaacc tttttaactg catcttcaat ggctttacct  13680
tcttcaggca agttcaatga caatttcaac atcattgcag cagacaagat agtggcgata  13740
gggttgacct tattctttgg caaatctgga gcggaaccat ggcatggttc gtacaaacca  13800
aatgcggtgt tcttgtctgg caaagaggcc aaggacgcag atggcaacaa acccaaggag  13860
cctgggataa cggaggcttc atcggagatg atatcaccaa acatgttgct ggtgattata  13920
ataccattta ggtgggttgg gttcttaact aggatcatgg cggcagaatc aatcaattga  13980
tgttgaactt tcaatgtagg gaattcgttc ttgatggttt cctccacagt ttttctccat  14040
aatcttgaag aggccaaaac attagcttta tccaaggacc aaataggcaa tggtggctca  14100
tgttgtaggg ccatgaaagc ggccattctt gtgattcttt gcacttctgg aacggtgtat  14160
tgttcactat cccaagcgac accatcacca tcgtcttcct ttctcttacc aaagtaaata  14220
cctcccacta attctctaac aacaacgaag tcagtacctt tagcaaattg tggcttgatt  14280
ggagataagt ctaaaagaga gtcggatgca aagttacatg gtcttaagtt ggcgtacaat  14340
tgaagttctt tacggatttt tagtaaacct tgttcaggtc taacactacc ggtaccccat  14400
ttaggaccac ccacagcacc taacaaaacg gcatcagcct tcttggaggc ttccagcgcc  14460
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aaatcgaact tgacattgga acgaacatca gaaatagctt taagaacctt aatggcttcg  14580
gctgtgattt cttgaccaac gtggtcacct ggcaaaacga cgatcttctt aggggcagac  14640
attacaatgg tatatccttg aaatatatat aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  14700
tgcagcttct caatgatatt cgaatacgct ttgaggagat acagcctaat atccgacaaa  14760
ctgttttaca gatttacgat cgtacttgtt acccatcatt gaattttgaa catccgaacc  14820
tgggagtttt ccctgaaaca gatagtatat ttgaacctgt ataataatat atagtctagc  14880
gctttacgga agacaatgta tgtatttcgg ttcctggaga aactattgca tctattgcat  14940
aggtaatctt gcacgtcgca tccccggttc attttctgcg tttccatctt gcacttcaat  15000
agcatatctt tgttaacgaa gcatctgtgc ttcattttgt agaacaaaaa tgcaacgcga  15060
gagcgctaat tttcaaaca aagaatctga gctgcatttt tacagaacag aaatgcaacg  15120
cgaaagcgct attttaccaa cgaagaatct gtgcttcatt tttgtaaaac aaaaatgcaa  15180
cgcgagagcg ctaattttc aaacaaagaa tctgagctgc atttttacag aacagaaatg  15240
caacgcgaga gcgctatttt accaacaaag aatctatact tcttttttgt tctacaaaaa  15300
tgcatcccga gagcgctatt tttctaacaa agcatcttag attacttttt ttctcctttg  15360
tgcgctctat aatgcagtct cttgataact ttttgcactg taggtccgtt aaggttagaa  15420
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ttttttgtc taaagagtaa tactagagat aaacataaaa aatgtagagg tcgagtttag  15780
atgcaagttc aaggagcgaa aggtggatgg gtaggttata tagggatata gcacagagat  15840
atatagcaaa gagatacttt tgagcaatgt ttgtggaagc ggtattcgca atattttagt  15900
```

agctcgttac agtccggtgc gtttttggtt ttttgaaagt gcgtcttcag agcgcttttg 15960

gttttcaaaa gcgctctgaa gttcctatac tttctagaga ataggaactt cggaatagga 16020

acttcaaagc gtttccgaaa acgagcgctt ccgaaaatgc aacgcgagct gcgcacatac 16080

agctcactgt tcacgtcgca cctatatctg cgtgttgcct gtatatatat atacatgaga 16140

agaacggcat agtgcgtgtt tatgcttaaa tgcgtactta tatgcgtcta tttatgtagg 16200

atgaaaggta gtctagtacc tcctgtgata ttatcccatt ccatgcgggg tatcgtatgc 16260

ttccttcagc actacccttt agctgttcta tatgctgcca ctcctcaatt ggattagtct 16320

catccttcaa tgctatcatt tcctttgata ttggatcata tgcatagtac cgagaaacta 16380

gaggatc 16387

<210> SEQ ID NO 24
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae CUP1 promoter

<400> SEQUENCE: 24

cccattaccg acatttgggc gctatacgtg catatgttca tgtatgtatc tgtatttaaa 60

acacttttgt attatttttc ctcatatatg tgtataggtt tatacggatg atttaattat 120

tacttcacca ccctttattt caggctgata tcttagcctt gttactagtt agaaaaagac 180

atttttgctg tcagtcactg tcaagagatt cttttgctgg catttcttct agaagcaaaa 240

agagcgatgc gtcttttccg ctgaaccgtt ccagcaaaaa agactaccaa cgcaatatgg 300

attgtcagaa tcatataaaa gagaagcaaa taactccttg tcttgtatca attgcattat 360

aatatcttct tgttagtgca atatcatata gaagtcatcg aaatagatat taagaaaaac 420

aaactgtaca atcaatcaat caatcatc 448

<210> SEQ ID NO 25
<211> LENGTH: 1716
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 25

atgttgacaa aagcaacaaa agaacaaaaa tcccttgtga aaaacagagg ggcggagctt 60

gttgttgatt gcttagtgga gcaaggtgtc acacatgtat ttggcattcc aggtgcaaaa 120

attgatgcgg tatttgacgc tttacaagat aaaggacctg aaattatcgt tgcccggcac 180

gaacaaaacg cagcattcat ggcccaagca gtcggccgtt taactggaaa accgggagtc 240

gtgttagtca catcaggacc gggtgcctct aacttggcaa caggcctgct gacagcgaac 300

actgaaggag accctgtcgt tgcgcttgct ggaaacgtga tccgtgcaga tcgtttaaaa 360

cggacacatc aatctttgga taatgcggcg ctattccagc cgattacaaa atacagtgta 420

gaagttcaag atgtaaaaaa tataccggaa gctgttacaa atgcatttag gatagcgtca 480

gcagggcagg ctggggccgc ttttgtgagc tttccgcaag atgttgtgaa tgaagtcaca 540

aatacgaaaa acgtgcgtgc tgttgcagcg ccaaaactcg gtcctgcagc agatgatgca 600

atcagtgcgg ccatagcaaa aatccaaaca gcaaaacttc ctgtcgtttt ggtcggcatg 660

aaaggcggaa gaccggaagc aattaaagcg gttcgcaagc ttttgaaaaa ggttcagctt 720

ccatttgttg aaacatatca agctgccggt accctttcta gagatttaga ggatcaatat 780

tttggccgta tcggtttgtt ccgcaaccag cctggcgatt tactgctaga gcaggcagat 840

```
gttgttctga cgatcggcta tgacccgatt gaatatgatc cgaaattctg gaatatcaat    900

ggagaccgga caattatcca tttagacgag attatcgctg acattgatca tgcttaccag    960

cctgatcttg aattgatcgg tgacattccg tccacgatca atcatatcga acacgatgct   1020

gtgaaagtgg aatttgcaga gcgtgagcag aaaatccttt ctgatttaaa acaatatatg   1080

catgaaggtg agcaggtgcc tgcagattgg aaatcagaca gagcgcaccc tcttgaaatc   1140

gttaaagagt tgcgtaatgc agtcgatgat catgttacag taacttgcga tatcggttcg   1200

cacgccattt ggatgtcacg ttatttccgc agctacgagc cgttaacatt aatgatcagt   1260

aacggtatgc aaacactcgg cgttgcgctt ccttgggcaa tcggcgcttc attggtgaaa   1320

ccgggagaaa aagtggtttc tgtctctggt gacggcggtt tcttattctc agcaatggaa   1380

ttagagacag cagttcgact aaaagcacca attgtacaca ttgtatggaa cgacagcaca   1440

tatgacatgg ttgcattcca gcaattgaaa aaatataacc gtacatctgc ggtcgatttc   1500

ggaaatatcg atatcgtgaa atatgcggaa agcttcggag caactggctt gcgcgtagaa   1560

tcaccagacc agctggcaga tgttctgcgt caaggcatga acgctgaagg tcctgtcatc   1620

atcgatgtcc cggttgacta cagtgataac attaatttag caagtgacaa gcttccgaaa   1680

gaattcgggg aactcatgaa aacgaaagct ctctag                             1716
```

<210> SEQ ID NO 26
<211> LENGTH: 571
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 26

```
Met Leu Thr Lys Ala Thr Lys Glu Gln Lys Ser Leu Val Lys Asn Arg
1               5                   10                  15

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

Val Phe Gly Ile Pro Gly Ala Lys Ile Asp Ala Val Phe Asp Ala Leu
        35                  40                  45

Gln Asp Lys Gly Pro Glu Ile Ile Val Ala Arg His Glu Gln Asn Ala
    50                  55                  60

Ala Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly Val
65                  70                  75                  80

Val Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly Leu
                85                  90                  95

Leu Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly Asn
            100                 105                 110

Val Ile Arg Ala Asp Arg Leu Lys Arg Thr His Gln Ser Leu Asp Asn
        115                 120                 125

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

Val Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala Ser
145                 150                 155                 160

Ala Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val Val
                165                 170                 175

Asn Glu Val Thr Asn Thr Lys Asn Val Arg Ala Val Ala Ala Pro Lys
            180                 185                 190

Leu Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys Ile
        195                 200                 205

Gln Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly Arg
```

```
    210                 215                 220

Pro Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln Leu
225                 230                 235                 240

Pro Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp Leu
                245                 250                 255

Glu Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro Gly
            260                 265                 270

Asp Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr Asp
        275                 280                 285

Pro Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg Thr
    290                 295                 300

Ile Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr Gln
305                 310                 315                 320

Pro Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His Ile
                325                 330                 335

Glu His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys Ile
            340                 345                 350

Leu Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro Ala
        355                 360                 365

Asp Trp Lys Ser Asp Arg Ala His Pro Leu Glu Ile Val Lys Glu Leu
    370                 375                 380

Arg Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly Ser
385                 390                 395                 400

His Ala Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu Thr
                405                 410                 415

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

Ala Ile Gly Ala Ser Leu Val Lys Pro Gly Glu Lys Val Val Ser Val
        435                 440                 445

Ser Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr Ala
    450                 455                 460

Val Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser Thr
465                 470                 475                 480

Tyr Asp Met Val Ala Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr Ser
                485                 490                 495

Ala Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser Phe
            500                 505                 510

Gly Ala Thr Gly Leu Arg Val Glu Ser Pro Asp Gln Leu Ala Asp Val
        515                 520                 525

Leu Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val Pro
    530                 535                 540

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

Glu Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
                565                 570
```

<210> SEQ ID NO 27
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae CYC1 terminator 2

<400> SEQUENCE: 27

```
ccgcaaatta aagccttcga gcgtcccaaa accttctcaa gcaaggtttt cagtataatg     60
```

```
ttacatgcgt acacgcgtct gtacagaaaa aaaagaaaaa tttgaaatat aaataacgtt    120

cttaatacta acataactat aaaaaaataa atagggacct agacttcagg ttgtctaact    180

ccttcctttt cggttagagc ggatgtgggg ggagggcgtg aatgtaagcg tgacataact    240

aattacatga                                                           250
```

<210> SEQ ID NO 28
<211> LENGTH: 1181
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae ILV5 promoter

<400> SEQUENCE: 28

```
taaaacctct agtggagtag tagatgtaat caatgaagcg gaagccaaaa gaccagagta     60

gaggcctata gaagaaactg cgataccttt tgtgatggct aaacaaacag acatcttttt    120

atatgttttt acttctgtat atcgtgaagt agtaagtgat aagcgaattt ggctaagaac    180

gttgtaagtg aacaagggac ctcttttgcc tttcaaaaaa ggattaaatg gagttaatca    240

ttgagattta gttttcgtta gattctgtat ccctaaataa ctcccttacc cgacgggaag    300

gcacaaaaga cttgaataat agcaaacggc cagtagccaa gaccaaataa tactagagtt    360

aactgatggt cttaaacagg cattacgtgg tgaactccaa gaccaatata caaaatatcg    420

ataagttatt cttgcccacc aatttaagga gcctacatca ggacagtagt accattcctc    480

agagaagagg tatacataac aagaaaatcg cgtgaacacc ttatataact tagcccgtta    540

ttgagctaaa aaaccttgca aaatttccta tgaataagaa tacttcagac gtgataaaaa    600

tttactttct aactcttctc acgctgcccc tatctgttct tccgctctac cgtgagaaat    660

aaagcatcga gtacggcagt tcgctgtcac tgaactaaaa caataaggct agttcgaatg    720

atgaacttgc ttgctgtcaa acttctgagt tgccgctgat gtgacactgt gacaataaat    780

tcaaaccggt tatagcggtc tcctccggta ccggttctgc cacctccaat agagctcagt    840

aggagtcaga acctctgcgg tggctgtcag tgactcatcc gcgtttcgta agttgtgcgc    900

gtgcacattt cgcccgttcc cgctcatctt gcagcaggcg gaaattttca tcacgctgta    960

ggacgcaaaa aaaaaataat taatcgtaca agaatcttgg aaaaaaaatt gaaaaatttt   1020

gtataaaagg gatgacctaa cttgactcaa tggcttttac acccagtatt ttccctttcc   1080

ttgtttgtta caattataga agcaagacaa aaacatatag acaacctatt cctaggagtt   1140

atattttttt accctaccag caatataagt aaaaaactag t                       1181
```

<210> SEQ ID NO 29
<211> LENGTH: 1017
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pf5.IlvC-JEA1 coding region

<400> SEQUENCE: 29

```
atgaaagttt tctacgataa agactgcgac ctgtcgatca tccaaggtaa gaaagttgcc     60

atcatcggct tcggttccca gggccacgct caagcactca acctgaagga ttccggcgta    120

gacgtgactg ttggcctgcc taaaggcttt gctgatgtag ccaaggctga agcccacggc    180

tttaaagtga ccgacgttgc tgcagccgtt gccggtgccg acttggtcat gatcctgatt    240

ccggacgagt tccagtccca gctgtacaag aacgaaatcg agccgaacat caagaagggc    300
```

```
gccactctgg ccttctccca cggcttcgcg atccactaca accaggttgt gcctcgtgcc    360

gacctcgacg tgatcatgat cgcgccgaag gctccaggcc acaccgtacg ttccgagttc    420

gtcaagggcg gaggtattcc tgacctgatc gcgatctacc aggacgtttc cggcaacgcc    480

aagaacgtcg ccctgtccta cgccgcaggc gtgggcggcg gccgtaccgg catcatcgaa    540

accaccttca aggacgagac tgaaaccgac ctgttcggtg agcaggctgt tctgtgtggc    600

ggtaccgtcg agctggtcaa agccggtttc gaaaccctgg ttgaagctgg ctacgctcca    660

gaaatggcct acttcgagtg cctgcacgaa ctgaagctga tcgttgacct catgtacgaa    720

ggcggtatcg ccaacatgaa ctactcgatc tccaacaacg ctgaatacgg cgagtacgtg    780

actggtccag aagtcatcaa cgccgaatcc cgtcaggcca tgcgcaatgc tctgaagcgc    840

atccaggacg gcgaatacgc gaagatgttc atcagcgaag gcgctaccgg ctacccatcg    900

atgaccgcca agcgtcgtaa caacgctgct cacggtatcg aaatcatcgg cgagcaactg    960

cgctcgatga tgccttggat cggtgccaac aaaatcgtcg acaaagccaa gaactaa      1017
```

```
<210> SEQ ID NO 30
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pf5.IlvC-JEA1 coding region

<400> SEQUENCE: 30

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
1               5                   10                  15

Lys Lys Val Ala Ile Ile Gly Phe Gly Ser Gln Gly His Ala Gln Ala
            20                  25                  30

Leu Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Pro Lys
        35                  40                  45

Gly Phe Ala Asp Val Ala Lys Ala Glu Ala His Gly Phe Lys Val Thr
    50                  55                  60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Ile
65                  70                  75                  80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
                85                  90                  95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
            100                 105                 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
        115                 120                 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
    130                 135                 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Val Ser Gly Asn Ala
145                 150                 155                 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr
                165                 170                 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
            180                 185                 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
        195                 200                 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
    210                 215                 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225                 230                 235                 240
```

```
Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
                245                 250                 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
            260                 265                 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
        275                 280                 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
    290                 295                 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305                 310                 315                 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
                325                 330                 335

Lys Asn


<210> SEQ ID NO 31
<211> LENGTH: 759
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae ILV5 terminator

<400> SEQUENCE: 31

ggccctgcag gcctatcaag tgctggaaac tttttctctt ggaatttttg caacatcaag     60

tcatagtcaa ttgaattgac ccaatttcac atttaagatt tttttttttt catccgacat    120

acatctgtac actaggaagc cctgtttttc tgaagcagct tcaaatatat atattttta    180

catatttatt atgattcaat gaacaatcta attaaatcga aaacaagaac cgaaacgcga    240

ataaataatt tatttagatg gtgacaagtg tataagtcct catcgggaca gctacgattt    300

ctctttcggt tttggctgag ctactggttg ctgtgacgca gcggcattag cgcggcgtta    360

tgagctaccc tcgtggcctg aaagatggcg ggaataaagc ggaactaaaa attactgact    420

gagccatatt gaggtcaatt tgtcaactcg tcaagtcacg tttggtggac ggcccctttc    480

caacgaatcg tatatactaa catgcgcgcg cttcctatat acacatatac atatatatat    540

atatatatat gtgtgcgtgt atgtgtacac ctgtatttaa tttccttact cgcgggtttt    600

tctttttct caattcttgg cttcctcttt ctcgagtata taatttttca ggtaaaattt    660

agtacgatag taaaatactt ctcgaactcg tcacatatac gtgtacataa tgtctgaacc    720

agctcaaaag aaacaaaagg ttgctaacaa ctctctaga                          759


<210> SEQ ID NO 32
<211> LENGTH: 643
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae FBA1 promoter

<400> SEQUENCE: 32

gaaatgaata acaatactga cagtactaaa taattgccta cttggcttca catacgttgc     60

atacgtcgat atagataata atgataatga cagcaggatt atcgtaatac gtaatagttg    120

aaaatctcaa aaatgtgtgg gtcattacgt aaataatgat aggaatggga ttcttctatt    180

tttccttttt ccattctagc agccgtcggg aaaacgtggc atcctctctt tcgggctcaa    240

ttggagtcac gctgccgtga gcatcctctc tttccatatc taacaactga gcacgtaacc    300

aatggaaaag catgagctta gcgttgctcc aaaaaagtat tggatggtta ataccatttg    360

tctgttctct tctgactttg actcctcaaa aaaaaaaaat ctacaatcaa cagatcgctt    420
```

```
caattacgcc ctcacaaaaa ctttttttcct tcttcttcgc ccacgttaaa ttttatccct    480

catgttgtct aacggatttc tgcacttgat ttattataaa aagacaaaga cataatactt    540

ctctatcaat ttcagttatt gttcttcctt gcgttattct tctgttcttc tttttctttt    600

gtcatatata accataacca agtaatacat attcaaatct aga                       643
```

<210> SEQ ID NO 33
<211> LENGTH: 1188
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 33

```
atgttgagaa ctcaagccgc cagattgatc tgcaactccc gtgtcatcac tgctaagaga     60

acctttgctt tggccacccg tgctgctgct tacagcagac cagctgcccg tttcgttaag    120

ccaatgatca ctacccgtgg tttgaagcaa atcaacttcg gtggtactgt tgaaaccgtc    180

tacgaaagag ctgactggcc aagagaaaag ttgttggact acttcaagaa cgacactttt    240

gctttgatcg gttacggttc ccaaggttac ggtcaaggtt tgaacttgag agacaacggt    300

ttgaacgtta tcattggtgt ccgtaaagat ggtgcttctt ggaaggctgc catcgaagac    360

ggttgggttc caggcaagaa cttgttcact gttgaagatg ctatcaagag aggtagttac    420

gttatgaact tgttgtccga tgccgctcaa tcagaaacct ggcctgctat caagccattg    480

ttgaccaagg gtaagacttt gtacttctcc cacggtttct ccccagtctt caaggacttg    540

actcacgttg aaccaccaaa ggacttagat gttatcttgg ttgctccaaa gggttccggt    600

agaactgtca gatctttgtt caaggaaggt cgtggtatta actcttctta cgccgtctgg    660

aacgatgtca ccggtaaggc tcacgaaaag gcccaagctt tggccgttgc cattggttcc    720

ggttacgttt accaaaccac tttcgaaaga gaagtcaact ctgacttgta cggtgaaaga    780

ggttgtttaa tgggtggtat ccacggtatg ttcttggctc aatacgacgt cttgagagaa    840

aacggtcact ccccatctga agctttcaac gaaaccgtcg aagaagctac ccaatctcta    900

tacccattga tcggtaagta cggtatggat tacatgtacg atgcttgttc caccaccgcc    960

agaagaggtg ctttggactg gtacccaatc ttcaagaatg ctttgaagcc tgttttccaa   1020

gacttgtacg aatctaccaa gaacggtacc gaaaccaaga gatctttgga attcaactct   1080

caacctgact acagagaaaa gctagaaaag gaattagaca ccatcagaaa catggaaatc   1140

tggaaggttg gtaaggaagt cagaaagttg agaccagaaa accaataa                1188
```

<210> SEQ ID NO 34
<211> LENGTH: 395
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 34

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

Thr Ala Lys Arg Thr Phe Ala Leu Ala Thr Arg Ala Ala Ala Tyr Ser
            20                  25                  30

Arg Pro Ala Ala Arg Phe Val Lys Pro Met Ile Thr Thr Arg Gly Leu
        35                  40                  45

Lys Gln Ile Asn Phe Gly Gly Thr Val Glu Thr Val Tyr Glu Arg Ala
    50                  55                  60

Asp Trp Pro Arg Glu Lys Leu Leu Asp Tyr Phe Lys Asn Asp Thr Phe
65                  70                  75                  80
```

```
Ala Leu Ile Gly Tyr Gly Ser Gln Gly Tyr Gly Gln Gly Leu Asn Leu
                85                  90                  95

Arg Asp Asn Gly Leu Asn Val Ile Ile Gly Val Arg Lys Asp Gly Ala
            100                 105                 110

Ser Trp Lys Ala Ala Ile Glu Asp Gly Trp Val Pro Gly Lys Asn Leu
        115                 120                 125

Phe Thr Val Glu Asp Ala Ile Lys Arg Gly Ser Tyr Val Met Asn Leu
    130                 135                 140

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

Leu Thr Lys Gly Lys Thr Leu Tyr Phe Ser His Gly Phe Ser Pro Val
                165                 170                 175

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

Leu Val Ala Pro Lys Gly Ser Gly Arg Thr Val Arg Ser Leu Phe Lys
        195                 200                 205

Glu Gly Arg Gly Ile Asn Ser Ser Tyr Ala Val Trp Asn Asp Val Thr
    210                 215                 220

Gly Lys Ala His Glu Lys Ala Gln Ala Leu Ala Val Ala Ile Gly Ser
225                 230                 235                 240

Gly Tyr Val Tyr Gln Thr Thr Phe Glu Arg Glu Val Asn Ser Asp Leu
                245                 250                 255

Tyr Gly Glu Arg Gly Cys Leu Met Gly Gly Ile His Gly Met Phe Leu
            260                 265                 270

Ala Gln Tyr Asp Val Leu Arg Glu Asn Gly His Ser Pro Ser Glu Ala
        275                 280                 285

Phe Asn Glu Thr Val Glu Glu Ala Thr Gln Ser Leu Tyr Pro Leu Ile
    290                 295                 300

Gly Lys Tyr Gly Met Asp Tyr Met Tyr Asp Ala Cys Ser Thr Thr Ala
305                 310                 315                 320

Arg Arg Gly Ala Leu Asp Trp Tyr Pro Ile Phe Lys Asn Ala Leu Lys
                325                 330                 335

Pro Val Phe Gln Asp Leu Tyr Glu Ser Thr Lys Asn Gly Thr Glu Thr
            340                 345                 350

Lys Arg Ser Leu Glu Phe Asn Ser Gln Pro Asp Tyr Arg Glu Lys Leu
        355                 360                 365

Glu Lys Glu Leu Asp Thr Ile Arg Asn Met Glu Ile Trp Lys Val Gly
    370                 375                 380

Lys Glu Val Arg Lys Leu Arg Pro Glu Asn Gln
385                 390                 395
```

```
<210> SEQ ID NO 35
<211> LENGTH: 244
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae CYC1 terminator

<400> SEQUENCE: 35

attaaagcct tcgagcgtcc caaaaccttc tcaagcaagg ttttcagtat aatgttacat     60

gcgtacacgc gtctgtacag aaaaaaaaga aaaatttgaa atataaataa cgttcttaat    120

actaacataa ctataaaaaa ataaataggg acctagactt caggttgtct aactccttcc    180

ttttcggtta gagcggatgt gggggagggg cgtgaatgta agcgtgacat aactaattac    240
```

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atga                                                                 244


<210> SEQ ID NO 36
<211> LENGTH: 570
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 36

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

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

Phe Gly Ile Pro Gly Ala Lys Ile Asp Ala Val Phe Asp Ala Leu Gln
        35                  40                  45

Asp Lys Gly Pro Glu Ile Ile Val Ala Arg His Glu Gln Asn Ala Ala
    50                  55                  60

Phe Met Ala Gln Ala Val Gly Arg Leu Thr Gly Lys Pro Gly Val Val
65                  70                  75                  80

Leu Val Thr Ser Gly Pro Gly Ala Ser Asn Leu Ala Thr Gly Leu Leu
                85                  90                  95

Thr Ala Asn Thr Glu Gly Asp Pro Val Val Ala Leu Ala Gly Asn Val
            100                 105                 110

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

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

Lys Asn Ile Pro Glu Ala Val Thr Asn Ala Phe Arg Ile Ala Ser Ala
145                 150                 155                 160

Gly Gln Ala Gly Ala Ala Phe Val Ser Phe Pro Gln Asp Val Val Asn
                165                 170                 175

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

Gly Pro Ala Ala Asp Asp Ala Ile Ser Ala Ala Ile Ala Lys Ile Gln
        195                 200                 205

Thr Ala Lys Leu Pro Val Val Leu Val Gly Met Lys Gly Gly Arg Pro
    210                 215                 220

Glu Ala Ile Lys Ala Val Arg Lys Leu Leu Lys Lys Val Gln Leu Pro
225                 230                 235                 240

Phe Val Glu Thr Tyr Gln Ala Ala Gly Thr Leu Ser Arg Asp Leu Glu
                245                 250                 255

Asp Gln Tyr Phe Gly Arg Ile Gly Leu Phe Arg Asn Gln Pro Gly Asp
            260                 265                 270

Leu Leu Leu Glu Gln Ala Asp Val Val Leu Thr Ile Gly Tyr Asp Pro
        275                 280                 285

Ile Glu Tyr Asp Pro Lys Phe Trp Asn Ile Asn Gly Asp Arg Thr Ile
    290                 295                 300

Ile His Leu Asp Glu Ile Ile Ala Asp Ile Asp His Ala Tyr Gln Pro
305                 310                 315                 320

Asp Leu Glu Leu Ile Gly Asp Ile Pro Ser Thr Ile Asn His Ile Glu
                325                 330                 335

His Asp Ala Val Lys Val Glu Phe Ala Glu Arg Glu Gln Lys Ile Leu
            340                 345                 350

Ser Asp Leu Lys Gln Tyr Met His Glu Gly Glu Gln Val Pro Ala Asp
        355                 360                 365
```

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Trp Lys Ser Asp Arg Ala His Pro Leu Glu Ile Val Lys Glu Leu Arg
    370                 375                 380

Asn Ala Val Asp Asp His Val Thr Val Thr Cys Asp Ile Gly Ser His
385                 390                 395                 400

Ala Ile Trp Met Ser Arg Tyr Phe Arg Ser Tyr Glu Pro Leu Thr Leu
                405                 410                 415

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

Ile Gly Ala Ser Leu Val Lys Pro Gly Glu Lys Val Val Ser Val Ser
        435                 440                 445

Gly Asp Gly Gly Phe Leu Phe Ser Ala Met Glu Leu Glu Thr Ala Val
    450                 455                 460

Arg Leu Lys Ala Pro Ile Val His Ile Val Trp Asn Asp Ser Thr Tyr
465                 470                 475                 480

Asp Met Val Ala Phe Gln Gln Leu Lys Lys Tyr Asn Arg Thr Ser Ala
                485                 490                 495

Val Asp Phe Gly Asn Ile Asp Ile Val Lys Tyr Ala Glu Ser Phe Gly
            500                 505                 510

Ala Thr Gly Leu Arg Val Glu Ser Pro Asp Gln Leu Ala Asp Val Leu
        515                 520                 525

Arg Gln Gly Met Asn Ala Glu Gly Pro Val Ile Ile Asp Val Pro Val
    530                 535                 540

Asp Tyr Ser Asp Asn Ile Asn Leu Ala Ser Asp Lys Leu Pro Lys Glu
545                 550                 555                 560

Phe Gly Glu Leu Met Lys Thr Lys Ala Leu
                565                 570


<210> SEQ ID NO 37
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Anaerostipes caccae

<400> SEQUENCE: 37

Met Glu Glu Cys Lys Met Ala Lys Ile Tyr Tyr Gln Glu Asp Cys Asn
1               5                   10                  15

Leu Ser Leu Leu Asp Gly Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser
            20                  25                  30

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

Ile Ile Gly Leu Tyr Glu Gly Ala Lys Glu Trp Lys Arg Ala Glu Glu
    50                  55                  60

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

Ile Ile Met Ile Leu Ile Asn Asp Glu Lys Gln Ala Thr Met Tyr Lys
                85                  90                  95

Asn Asp Ile Glu Pro Asn Leu Glu Ala Gly Asn Met Leu Met Phe Ala
            100                 105                 110

His Gly Phe Asn Ile His Phe Gly Cys Ile Val Pro Pro Lys Asp Val
        115                 120                 125

Asp Val Thr Met Ile Ala Pro Lys Gly Pro Gly His Thr Val Arg Ser
    130                 135                 140

Glu Tyr Glu Glu Gly Lys Gly Val Pro Cys Leu Val Ala Val Glu Gln
145                 150                 155                 160

Asp Ala Thr Gly Lys Ala Leu Asp Met Ala Leu Ala Tyr Ala Leu Ala
                165                 170                 175
```

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Ile Gly Gly Ala Arg Ala Gly Val Leu Glu Thr Thr Phe Arg Thr Glu
            180                 185                 190

Thr Glu Thr Asp Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Val
        195                 200                 205

Cys Ala Leu Met Gln Ala Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr
    210                 215                 220

Asp Pro Arg Asn Ala Tyr Phe Glu Cys Ile His Glu Met Lys Leu Ile
225                 230                 235                 240

Val Asp Leu Ile Tyr Gln Ser Gly Phe Ser Gly Met Arg Tyr Ser Ile
                245                 250                 255

Ser Asn Thr Ala Glu Tyr Gly Asp Tyr Ile Thr Gly Pro Lys Ile Ile
            260                 265                 270

Thr Glu Asp Thr Lys Lys Ala Met Lys Lys Ile Leu Ser Asp Ile Gln
        275                 280                 285

Asp Gly Thr Phe Ala Lys Asp Phe Leu Val Asp Met Ser Asp Ala Gly
    290                 295                 300

Ser Gln Val His Phe Lys Ala Met Arg Lys Leu Ala Ser Glu His Pro
305                 310                 315                 320

Ala Glu Val Val Gly Glu Glu Ile Arg Ser Leu Tyr Ser Trp Ser Asp
                325                 330                 335

Glu Asp Lys Leu Ile Asn Asn
            340


<210> SEQ ID NO 38
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Anaerostipes caccae

<400> SEQUENCE: 38

Met Glu Glu Cys Lys Met Ala Lys Ile Tyr Tyr Gln Glu Asp Cys Asn
1               5                   10                  15

Leu Ser Leu Leu Asp Gly Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser
            20                  25                  30

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

Ile Ile Gly Leu Tyr Glu Gly Ala Lys Asp Trp Lys Arg Ala Glu Glu
    50                  55                  60

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

Ile Ile Met Ile Leu Ile Asn Asp Glu Lys Gln Ala Thr Met Tyr Lys
                85                  90                  95

Asn Asp Ile Glu Pro Asn Leu Glu Ala Gly Asn Met Leu Met Phe Ala
            100                 105                 110

His Gly Phe Asn Ile His Phe Gly Cys Ile Val Pro Pro Lys Asp Val
        115                 120                 125

Asp Val Thr Met Ile Ala Pro Lys Gly Pro Gly His Thr Val Arg Ser
    130                 135                 140

Glu Tyr Glu Glu Gly Lys Gly Val Pro Cys Leu Val Ala Val Glu Gln
145                 150                 155                 160

Asp Ala Thr Gly Lys Ala Leu Asp Met Ala Leu Ala Tyr Ala Leu Ala
                165                 170                 175

Ile Gly Gly Ala Arg Ala Gly Val Leu Glu Thr Thr Phe Arg Thr Glu
            180                 185                 190

Thr Glu Thr Asp Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Val
```

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        195                 200                 205

Cys Ala Leu Met Gln Ala Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr
    210                 215                 220

Asp Pro Arg Asn Ala Tyr Phe Glu Cys Ile His Glu Met Lys Leu Ile
225                 230                 235                 240

Val Asp Leu Ile Tyr Gln Ser Gly Phe Ser Gly Met Arg Tyr Ser Ile
                245                 250                 255

Ser Asn Thr Ala Glu Tyr Gly Asp Tyr Ile Thr Gly Pro Lys Ile Ile
            260                 265                 270

Thr Glu Asp Thr Lys Lys Ala Met Lys Lys Ile Leu Ser Asp Ile Gln
        275                 280                 285

Asp Gly Thr Phe Ala Lys Asp Phe Leu Val Asp Met Ser Asp Ala Gly
    290                 295                 300

Ser Gln Val His Phe Lys Ala Met Arg Lys Leu Ala Ser Glu His Pro
305                 310                 315                 320

Ala Glu Val Val Gly Glu Glu Ile Arg Ser Leu Tyr Ser Trp Ser Asp
                325                 330                 335

Glu Asp Lys Leu Ile Asn Asn
            340


<210> SEQ ID NO 39
<211> LENGTH: 15539
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: pLH468

<400> SEQUENCE: 39

tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca     60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg    120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc    180

accataaatt cccgttttaa gagcttggtg agcgctagga gtcactgcca ggtatcgttt    240

gaacacggca ttagtcaggg aagtcataac acagtccttt cccgcaattt tctttttcta    300

ttactcttgg cctcctctag tacactctat atttttttat gcctcggtaa tgattttcat    360

tttttttttt ccacctagcg gatgactctt tttttttctt agcgattggc attatcacat    420

aatgaattat acattatata aagtaatgtg atttcttcga agaatatact aaaaaatgag    480

caggcaagat aaacgaaggc aaagatgaca gagcagaaag ccctagtaaa gcgtattaca    540

aatgaaacca agattcagat tgcgatctct ttaaagggtg gtcccctagc gatagagcac    600

tcgatcttcc cagaaaaaga ggcagaagca gtagcagaac aggccacaca atcgcaagtg    660

attaacgtcc acacaggtat agggtttctg gaccatatga tacatgctct ggccaagcat    720

tccggctggt cgctaatcgt tgagtgcatt ggtgacttac acatagacga ccatcacacc    780

actgaagact gcgggattgc tctcggtcaa gcttttaaag aggccctagg ggccgtgcgt    840

ggagtaaaaa ggtttggatc aggatttgcg cctttggatg aggcactttc cagagcggtg    900

gtagatcttt cgaacaggcc gtacgcagtt gtcgaacttg gtttgcaaag ggagaaagta    960

ggagatctct cttgcgagat gatcccgcat tttcttgaaa gctttgcaga ggctagcaga   1020

attaccctcc acgttgattg tctgcgaggc aagaatgatc atcaccgtag tgagagtgcg   1080

ttcaaggctc ttgcggttgc cataagagaa gccacctcgc ccaatggtac caacgatgtt   1140

ccctccacca aaggtgttct tatgtagtga caccgattat ttaaagctgc agcatacgat   1200
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atatatacat gtgtatatat gtatacctat gaatgtcagt aagtatgtat acgaacagta   1260
tgatactgaa gatgacaagg taatgcatca ttctatacgt gtcattctga acgaggcgcg   1320
ctttcctttt ttcttttttgc tttttctttt tttttctctt gaactcgacg gatctatgcg   1380
gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggaaat tgtaagcgtt   1440
aatattttgt taaaattcgc gttaaatttt tgttaaatca gctcattttt taaccaatag   1500
gccgaaatcg gcaaaatccc ttataaatca aaagaataga ccgagatagg gttgagtgtt   1560
gttccagttt ggaacaagag tccactatta aagaacgtgg actccaacgt caaagggcga   1620
aaaaccgtct atcagggcga tggcccacta cgtgaaccat caccctaatc aagttttttg   1680
gggtcgaggt gccgtaaagc actaaatcgg aaccctaaag ggagcccccg atttagagct   1740
tgacggggaa agccggcgaa cgtggcgaga aaggaaggga agaaagcgaa aggagcgggc   1800
gctagggcgc tggcaagtgt agcggtcacg ctgcgcgtaa ccaccacacc cgccgcgctt   1860
aatgcgccgc tacagggcgc gtccattcgc cattcaggct gcgcaactgt tgggaagggc   1920
gcggtgcggg cctcttcgct attacgccag ctggcgaaag ggggatgtgc tgcaaggcga   1980
ttaagttggg taacgccagg gttttcccag tcacgacgtt gtaaaacgac ggccagtgag   2040
cgcgcgtaat acgactcact atagggcgaa ttgggtaccg ggccccccct cgaggtcgac   2100
ggcgcgccac tggtagagag cgactttgta tgccccaatt gcgaaacccg cgatatcctt   2160
ctcgattctt tagtacccga ccaggacaag gaaaaggagg tcgaaacgtt tttgaagaaa   2220
caagaggaac tacacggaag ctctaaagat ggcaaccagc cagaaactaa gaaaatgaag   2280
ttgatggatc caactggcac cgctggcttg aacaacaata ccagccttcc aacttctgta   2340
aataacggcg gtacgccagt gccaccagta ccgttacctt tcggtatacc tcctttcccc   2400
atgtttccaa tgcccttcat gcctccaacg gctactatca caaatcctca tcaagctgac   2460
gcaagcccta agaaatgaat aacaatactg acagtactaa ataattgcct acttggcttc   2520
acatacgttg catacgtcga tatagataat aatgataatg acagcaggat tatcgtaata   2580
cgtaatagct gaaaatctca aaaatgtgtg ggtcattacg taaataatga taggaatggg   2640
attcttctat ttttcctttt tccattctag cagccgtcgg gaaaacgtgg catcctctct   2700
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agcacgtaac caatggaaaa gcatgagctt agcgttgctc caaaaaagta ttggatggtt   2820
aataccattt gtctgttctc ttctgacttt gactcctcaa aaaaaaaaat ctacaatcaa   2880
cagatcgctt caattacgcc ctcacaaaaa cttttttcct tcttcttcgc ccacgttaaa   2940
ttttatccct catgttgtct aacggatttc tgcacttgat ttattataaa aagacaaaga   3000
cataatactt ctctatcaat ttcagttatt gttcttcctt gcgttattct tctgttcttc   3060
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caaaaaaact cttaaagact taagaaatcg tagttctgtt tacgattcaa tggttaaatc   3180
acctaatcgt gctatgttgc gtgcaactgg tatgcaagat gaagactttg aaaaacctat   3240
cgtcggtgtc atttcaactt gggctgaaaa cacaccttgt aatatccact tacatgactt   3300
tggtaaacta gccaaagtcg gtgttaagga agctggtgct tggccagttc agttcggaac   3360
aatcacggtt tctgatggaa tcgccatggg aacccaagga atgcgtttct ccttgacatc   3420
tcgtgatatt attgcagatt ctattgaagc agccatggga ggtcataatg cggatgcttt   3480
tgtagccatt ggcggttgtg ataaaaacat gcccggttct gttatcgcta tggctaacat   3540
ggatatccca gccatttttg cttacggcgg aacaattgca cctggtaatt tagacggcaa   3600
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agatatcgat ttagtctctg tctttgaagg tgtcggccat tggaaccacg gcgatatgac   3660
caaagaagaa gttaaagctt tggaatgtaa tgcttgtccc ggtcctggag gctgcggtgg   3720
tatgtatact gctaacacaa tggcgacagc tattgaagtt ttgggactta gccttccggg   3780
ttcatcttct cacccggctg aatccgcaga aaagaaagca gatattgaag aagctggtcg   3840
cgctgttgtc aaaatgctcg aaatgggctt aaaaccttct gacattttaa cgcgtgaagc   3900
ttttgaagat gctattactg taactatggc tctgggaggt tcaaccaact caacccttca   3960
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ccaagaaaaa gttcctcatt tggctgattt gaaaccttct ggtcaatatg tattccaaga   4080
cctttacaag gtcggagggg taccagcagt tatgaaatat ctccttaaaa atggcttcct   4140
tcatggtgac cgtatcactt gtactggcaa aacagtcgct gaaaatttga aggcttttga   4200
tgatttaaca cctggtcaaa aggttattat gccgcttgaa aatcctaaac gtgaagatgg   4260
tccgctcatt attctccatg gtaacttggc tccagacggt gccgttgcca aagtttctgg   4320
tgtaaaagtg cgtcgtcatg tcggtcctgc taaggtcttt aattctgaag aagaagccat   4380
tgaagctgtc ttgaatgatg atattgttga tggtgatgtt gttgtcgtac gttttgtagg   4440
accaaagggc ggtcctggta tgcctgaaat gctttccctt tcatcaatga ttgttggtaa   4500
agggcaaggt gaaaaagttg cccttctgac agatggccgc ttctcaggtg gtacttatgg   4560
tcttgtcgtg ggtcatatcg ctcctgaagc acaagatggc ggtccaatcg cctacctgca   4620
aacaggagac atagtcacta ttgaccaaga cactaaggaa ttacactttg atatctccga   4680
tgaagagtta aaacatcgtc aagagaccat tgaattgcca ccgctctatt cacgcggtat   4740
ccttggtaaa tatgctcaca tcgtttcgtc tgcttctagg ggagccgtaa cagacttttg   4800
gaagcctgaa gaaactggca aaaaatgttg tcctggttgc tgtggttaag cggccgcgtt   4860
aattcaaatt aattgatata gtttttaat gagtattgaa tctgtttaga aataatggaa   4920
tattattttt atttatttat ttatattatt ggtcggctct ttcttctga aggtcaatga   4980
caaaatgata tgaaggaaat aatgatttct aaaattttac aacgtaagat atttttacaa   5040
aagcctagct catcttttgt catgcactat tttactcacg cttgaaatta acggccagtc   5100
cactgcggag tcatttcaaa gtcatcctaa tcgatctatc gtttttgata gctcattttg   5160
gagttcgcga ttgtcttctg ttattcacaa ctgttttaat tttatttca ttctggaact   5220
cttcgagttc tttgtaaagt ctttcatagt agcttacttt atcctccaac atatttaact   5280
tcatgtcaat ttcggctctt aaattttcca catcatcaag ttcaacatca tcttttaact   5340
tgaatttatt ctctagctct tccaaccaag cctcattgct ccttgattta ctggtgaaaa   5400
gtgatacact ttgcgcgcaa tccaggtcaa aactttcctg caaagaattc accaatttct   5460
cgacatcata gtacaatttg ttttgttctc ccatcacaat ttaatatacc tgatggattc   5520
ttatgaagcg ctgggtaatg gacgtgtcac tctacttcgc ctttttccct actcctttta   5580
gtacggaaga caatgctaat aaataagagg gtaataataa tattattaat cggcaaaaaa   5640
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<210> SEQ ID NO 40
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Codon-optimized polynucleotide sequence-Horse-
liver ADH

<400> SEQUENCE: 40

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<210> SEQ ID NO 41
<211> LENGTH: 1023
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae TDH3 promoter

<400> SEQUENCE: 41

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aaa                                                                1023


<210> SEQ ID NO 42
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer OT1068

<400> SEQUENCE: 42

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<210> SEQ ID NO 43
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<220> FEATURE:
<223> OTHER INFORMATION: Primer OT1067

<400> SEQUENCE: 43

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLH467

<400> SEQUENCE: 44

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gctcatgttg tagggccatg aaagcggcca ttcttgtgat tctttgcact tctggaacgg   4920
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aaatacctcc cactaattct ctgacaacaa cgaagtcagt acctttagca aattgtggct   5040
tgattggaga taagtctaaa agagagtcgg atgcaaagtt acatggtctt aagttggcgt   5100
acaattgaag ttctttacgg atttttagta aaccttgttc aggtctaaca ctaccggtac   5160
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gcgcctcatc tggaagtggg acacctgtag catcgatagc agcaccacca attaaatgat   5280
tttcgaaatc gaacttgaca ttggaacgaa catcagaaat agctttaaga accttaatgg   5340
```

```
cttcggctgt gatttcttga ccaacgtggt cacctggcaa aacgacgatc ttcttagggg   5400
cagacatagg ggcagacatt agaatggtat atccttgaaa tatatatata tattgctgaa   5460
atgtaaaagg taagaaaagt tagaaagtaa gacgattgct aaccacctat tggaaaaaac   5520
aataggtcct taaataatat tgtcaacttc aagtattgtg atgcaagcat ttagtcatga   5580
acgcttctct attctatatg aaaagccggt tccggcctct cacctttcct ttttctccca   5640
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caattacttg ttgagaaata gagtataatt atcctataaa tataacgttt ttgaacacac   5940
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tttcggttaa cacatgcagt gatgcacgcg cgatggtgct aagttacata tatatatata   7140
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attctcttcc tccttcttgt cctttcttaa ttctgttgta attaccttcc tttgtaattt   7560
tttttgtaat tattcttctt aataatccaa acaaacacac atattacaat agctagctga   7620
ggatgaaggc attagtttat catggggatc acaaaatttc gttagaagac aaaccaaaac   7680
```

```
ccactctgca gaaaccaaca gacgttgtgg ttagggtgtt gaaaacaaca atttgcggta   7740

ctgacttggg aatatacaaa ggtaagaatc ctgaagtggc agatggcaga atcctgggtc   7800

atgagggcgt tggcgtcatt gaagaagtgg gcgaatccgt gacacaattc aaaaaggggg   7860

ataaagtttt aatctcctgc gttactagct gtggatcgtg tgattattgc aagaagcaac   7920

tgtattcaca ctgtagagac ggtggctgga ttttaggtta catgatcgac ggtgtccaag   7980

ccgaatacgt cagaatacca catgctgaca attcattgta taagatcccg caaactatcg   8040

atgatgaaat tgcagtacta ctgtccgata ttttacctac tggacatgaa attggtgttc   8100

aatatggtaa cgttcaacca ggcgatgctg tagcaattgt aggagcaggt cctgttggaa   8160

tgtcagtttt gttaactgct caattttact cgcctagtac cattattgtt atcgacatgg   8220

acgaaaaccg tttacaatta gcgaaggagc ttggggccac acacactatt aactccggta   8280

ctgaaaatgt tgtcgaagct gtgcatcgta tagcagccga aggagtggat gtagcaatag   8340

aagctgttgg tatacccgca acctgggaca tctgtcagga aattgtaaaa cccggcgctc   8400

atattgccaa cgtgggagtt catggtgtta aggtggactt tgaaattcaa aagttgtgga   8460

ttaagaatct aaccatcacc actggtttgg ttaacactaa tactacccca atgttgatga   8520

aggtagcctc tactgataaa ttgcctttaa agaaaatgat tactcacagg tttgagttag   8580

ctgaaatcga acacgcatat caggttttct tgaatggcgc taaagaaaaa gctatgaaga   8640

ttattctatc taatgcaggt gccgcctaat taattaagag taagcgaatt tcttatgatt   8700

tatgattttt attattaaat aagttataaa aaaaataagt gtatacaaat tttaaagtga   8760

ctcttaggtt ttaaaacgaa aattcttatt cttgagtaac tctttcctgt aggtcaggtt   8820

gctttctcag gtatagcatg aggtcgctct tattgaccac acctctaccg gcatgccgag   8880

caaatgcctg caaatcgctc cccatttcac ccaattgtag atatgctaac tccagcaatg   8940

agttgatgaa tctcggtgtg tattttatgt cctcagagga caacacctgt ggta         8994
```

```
<210> SEQ ID NO 46
<211> LENGTH: 753
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae GPM1 promoter

<400> SEQUENCE: 46
```

```
gcatgcttgc atttagtcgt gcaatgtatg actttaagat ttgtgagcag gaagaaaagg     60

gagaatcttc taacgataaa cccttgaaaa actgggtaga ctacgctatg ttgagttgct    120

acgcaggctg cacaattaca cgagaatgct cccgcctagg atttaaggct aagggacgtg    180

caatgcagac gacagatcta aatgaccgtg tcggtgaagt gttcgccaaa cttttcggtt    240

aacacatgca gtgatgcacg cgcgatggtg ctaagttaca tatatatata tatagccata    300

gtgatgtcta agtaaccttt atggtatatt tcttaatgtg gaaagatact agcgcgcgca    360

cccacacaca agcttcgtct tttcttgaag aaaagaggaa gctcgctaaa tgggattcca    420

ctttccgttc cctgccagct gatggaaaaa ggttagtgga acgatgaaga ataaaaagag    480

agatccactg aggtgaaatt tcagctgaca gcgagtttca tgatcgtgat gaacaatggt    540

aacgagttgt ggctgttgcc agggagggtg gttctcaact tttaatgtat ggccaaatcg    600

ctacttgggt ttgttatata acaaagaaga aataatgaac tgattctctt cctccttctt    660

gtcctttctt aattctgttg taattacctt cctttgtaat ttttttgta attattcttc    720

ttaataatcc aaacaaacac acatattaca ata                                 753
```

<210> SEQ ID NO 47
<211> LENGTH: 1047
<212> TYPE: DNA
<213> ORGANISM: Achromobacter xylosoxidans

<400> SEQUENCE: 47

```
atgaaagctc tggtttatca cggtgaccac aagatctcgc ttgaagacaa gcccaagccc     60

acccttcaaa agcccacgga tgtagtagta cgggttttga agaccacgat ctgcggcacg    120

gatctcggca tctacaaagg caagaatcca gaggtcgccg acgggcgcat cctgggccat    180

gaaggggtag gcgtcatcga ggaagtgggc gagagtgtca cgcagttcaa gaaaggcgac    240

aaggtcctga tttcctgcgt cacttcttgc ggctcgtgcg actactgcaa gaagcagctt    300

tactcccatt gccgcgacgg cgggtggatc ctgggttaca tgatcgatgg cgtgcaggcc    360

gaatacgtcc gcatcccgca tgccgacaac agcctctaca agatccccca gacaattgac    420

gacgaaatcg ccgtcctgct gagcgacatc ctgcccaccg gccacgaaat cggcgtccag    480

tatgggaatg tccagccggg cgatgcggtg gctattgtcg gcgcgggccc cgtcggcatg    540

tccgtactgt tgaccgccca gttctactcc ccctcgacca tcatcgtgat cgacatggac    600

gagaatcgcc tccagctcgc caaggagctc ggggcaacgc acaccatcaa ctccggcacg    660

gagaacgttg tcgaagccgt gcataggatt gcggcagagg gagtcgatgt tgcgatcgag    720

gcggtgggca taccggcgac ttgggacatc tgccaggaga tcgtcaagcc cggcgcgcac    780

atcgccaacg tcggcgtgca tggcgtcaag gttgacttcg agattcagaa gctctggatc    840

aagaacctga cgatcaccac gggactggtg aacacgaaca cgacgcccat gctgatgaag    900

gtcgcctcga ccgacaagct tccgttgaag aagatgatta cccatcgctt cgagctggcc    960

gagatcgagc acgcctatca ggtattcctc aatggcgcca aggagaaggc gatgaagatc   1020

atcctctcga acgcaggcgc tgcctga                                       1047
```

<210> SEQ ID NO 48
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Achromobacter xylosoxidans

<400> SEQUENCE: 48

```
Met Lys Ala Leu Val Tyr His Gly Asp His Lys Ile Ser Leu Glu Asp
1               5                   10                  15

Lys Pro Lys Pro Thr Leu Gln Lys Pro Thr Asp Val Val Val Arg Val
            20                  25                  30

Leu Lys Thr Thr Ile Cys Gly Thr Asp Leu Gly Ile Tyr Lys Gly Lys
        35                  40                  45

Asn Pro Glu Val Ala Asp Gly Arg Ile Leu Gly His Glu Gly Val Gly
    50                  55                  60

Val Ile Glu Glu Val Gly Glu Ser Val Thr Gln Phe Lys Lys Gly Asp
65                  70                  75                  80

Lys Val Leu Ile Ser Cys Val Thr Ser Cys Gly Ser Cys Asp Tyr Cys
                85                  90                  95

Lys Lys Gln Leu Tyr Ser His Cys Arg Asp Gly Gly Trp Ile Leu Gly
            100                 105                 110

Tyr Met Ile Asp Gly Val Gln Ala Glu Tyr Val Arg Ile Pro His Ala
        115                 120                 125

Asp Asn Ser Leu Tyr Lys Ile Pro Gln Thr Ile Asp Asp Glu Ile Ala
    130                 135                 140
```

```
Val Leu Leu Ser Asp Ile Leu Pro Thr Gly His Glu Ile Gly Val Gln
145                 150                 155                 160

Tyr Gly Asn Val Gln Pro Gly Asp Ala Val Ala Ile Val Gly Ala Gly
                165                 170                 175

Pro Val Gly Met Ser Val Leu Leu Thr Ala Gln Phe Tyr Ser Pro Ser
            180                 185                 190

Thr Ile Ile Val Ile Asp Met Asp Glu Asn Arg Leu Gln Leu Ala Lys
        195                 200                 205

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

Glu Ala Val His Arg Ile Ala Ala Glu Gly Val Asp Val Ala Ile Glu
225                 230                 235                 240

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

Pro Gly Ala His Ile Ala Asn Val Gly Val His Gly Val Lys Val Asp
            260                 265                 270

Phe Glu Ile Gln Lys Leu Trp Ile Lys Asn Leu Thr Ile Thr Thr Gly
        275                 280                 285

Leu Val Asn Thr Asn Thr Thr Pro Met Leu Met Lys Val Ala Ser Thr
    290                 295                 300

Asp Lys Leu Pro Leu Lys Lys Met Ile Thr His Arg Phe Glu Leu Ala
305                 310                 315                 320

Glu Ile Glu His Ala Tyr Gln Val Phe Leu Asn Gly Ala Lys Glu Lys
                325                 330                 335

Ala Met Lys Ile Ile Leu Ser Asn Ala Gly Ala Ala
            340                 345
```

<210> SEQ ID NO 49
<211> LENGTH: 316
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Saccharomyces cerevisiae ADH1 terminator

<400> SEQUENCE: 49

```
gagtaagcga atttcttatg atttatgatt tttattatta aataagttat aaaaaaaata     60

agtgtataca aattttaaag tgactcttag gttttaaaac gaaaattctt attcttgagt    120

aactctttcc tgtaggtcag gttgctttct caggtatagc atgaggtcgc tcttattgac    180

cacacctcta ccggcatgcc gagcaaatgc ctgcaaatcg ctccccattt cacccaattg    240

tagatatgct aactccagca atgagttgat gaatctcggt gtgtatttta tgtcctcaga    300

ggacaacacc tgtggt                                                     316
```

<210> SEQ ID NO 50
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer OT1074

<400> SEQUENCE: 50

```
cacacatatt acaatagcta gctgaggatg aaagctctg                            39
```

<210> SEQ ID NO 51
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

```
<220> FEATURE:
<223> OTHER INFORMATION: Primer OT1075

<400> SEQUENCE: 51

cagagctttc atcctcagct agctattgta atatgtgtg                             39


<210> SEQ ID NO 52
<211> LENGTH: 9075
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLH435

<400> SEQUENCE: 52

ctagttctag agcggccgcc accgcggtgg agctccagct tttgttccct ttagtgaggg      60

ttaattgcgc gcttggcgta atcatggtca tagctgtttc ctgtgtgaaa ttgttatccg     120

ctcacaattc cacacaacat aggagccgga agcataaagt gtaaagcctg gggtgcctaa     180

tgagtgaggt aactcacatt aattgcgttg cgctcactgc ccgctttcca gtcgggaaac     240

ctgtcgtgcc agctgcatta atgaatcggc caacgcgcgg ggagaggcgg tttgcgtatt     300

gggcgctctt ccgcttcctc gctcactgac tcgctgcgct cggtcgttcg gctgcggcga     360

gcggtatcag ctcactcaaa ggcggtaata cggttatcca cagaatcagg ggataacgca     420

ggaaagaaca tgtgagcaaa aggccagcaa aaggccagga accgtaaaaa ggccgcgttg     480

ctggcgtttt tccataggct ccgcccccct gacgagcatc acaaaaatcg acgctcaagt     540

cagaggtggc gaaacccgac aggactataa agataccagg cgtttccccc tggaagctcc     600

ctcgtgcgct ctcctgttcc gaccctgccg cttaccggat acctgtccgc ctttctccct     660

tcgggaagcg tggcgctttc tcatagctca cgctgtaggt atctcagttc ggtgtaggtc     720

gttcgctcca agctgggctg tgtgcacgaa ccccccgttc agcccgaccg ctgcgcctta     780

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gccactggta acaggattag cagagcgagg tatgtaggcg gtgctacaga gttcttgaag     900

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gatcctttga tcttttctac ggggtctgac gctcagtgga acgaaaactc acgttaaggg    1140

attttggtca tgagattatc aaaaaggatc ttcacctaga tccttttaaa ttaaaaatga    1200

agttttaaat caatctaaag tatatatgag taaacttggt ctgacagtta ccaatgctta    1260

atcagtgagg cacctatctc agcgatctgt ctatttcgtt catccatagt tgcctgactc    1320

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gctacaggca tcgtggtgtc acgctcgtcg tttggtatgg cttcattcag ctccggttcc    1620

caacgatcaa ggcgagttac atgatccccc atgttgtgca aaaaagcggt tagctccttc    1680

ggtcctccga tcgttgtcag aagtaagttg gccgcagtgt tatcactcat ggttatggca    1740

gcactgcata attctcttac tgtcatgcca tccgtaagat gcttttctgt gactggtgag    1800

tactcaacca agtcattctg agaatagtgt atgcggcgac cgagttgctc ttgcccggcg    1860
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tcaatacggg ataataccgc gccacatagc agaactttaa aagtgctcat cattggaaaa   1920
cgttcttcgg ggcgaaaact ctcaaggatc ttaccgctgt tgagatccag ttcgatgtaa   1980
cccactcgtg cacccaactg atcttcagca tcttttactt tcaccagcgt ttctgggtga   2040
gcaaaaacag gaaggcaaaa tgccgcaaaa aagggaataa gggcgacacg gaaatgttga   2100
atactcatac tcttcctttt tcaatattat tgaagcattt atcagggtta ttgtctcatg   2160
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ccccgaaaag tgccacctga acgaagcatc tgtgcttcat tttgtagaac aaaaatgcaa   2280
cgcgagagcg ctaatttttc aaacaaagaa tctgagctgc atttttacag aacagaaatg   2340
caacgcgaaa gcgctatttt accaacgaag aatctgtgct tcatttttgt aaaacaaaaa   2400
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ctttgtgcgc tctataatgc agtctcttga taactttttg cactgtaggt ccgttaaggt   2640
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gattatattc tataccgatg tggattgcgc atactttgtg aacagaaagt gatagcgttg   2820
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cgtataggaa atgtttacat tttcgtattg ttttcgattc actctatgaa tagttcttac   2940
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tttagatgca agttcaagga gcgaaaggtg gatgggtagg ttatataggg atatagcaca   3060
gagatatata gcaaagagat acttttgagc aatgtttgtg gaagcggtat tcgcaatatt   3120
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ttttggtttt caaaagcgct ctgaagttcc tatactttct agagaatagg aacttcggaa   3240
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catacagctc actgttcacg tcgcacctat atctgcgtgt tgcctgtata tatatataca   3360
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gtaggatgaa aggtagtcta gtacctcctg tgatattatc ccattccatg cggggtatcg   3480
tatgcttcct tcagcactac cctttagctg ttctatatgc tgccactcct caattggatt   3540
agtctcatcc ttcaatgcta tcatttcctt tgatattgga tcatactaag aaaccattat   3600
tatcatgaca ttaacctata aaaataggcg tatcacgagg ccctttcgtc tcgcgcgttt   3660
cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca cagcttgtct   3720
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gaaatgcttc aagaaggtat tgacttaaac tccatcaaat ggtcaggtca ttgagtgttt   3960
tttatttgtt gtattttttt ttttttagag aaaatcctcc aatatcaaat taggaatcgt   4020
agtttcatga ttttctgtta cacctaactt tttgtgtggt gccctcctcc ttgtcaatat   4080
taatgttaaa gtgcaattct tttccttat cacgttgagc cattagtatc aatttgctta   4140
cctgtattcc tttactatcc tcctttttct ccttcttgat aaatgtatgt agattgcgta   4200
tatagtttcg tctaccctat gaacatattc cattttgtaa tttcgtgtcg tttctattat   4260
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```
gaatttcatt tataaagttt atgtacaaat atcataaaaa aagagaatct ttttaagcaa   4320
ggattttctt aacttcttcg gcgacagcat caccgacttc ggtggtactg ttggaaccac   4380
ctaaatcacc agttctgata cctgcatcca aaaccttttt aactgcatct tcaatggcct   4440
taccttcttc aggcaagttc aatgacaatt tcaacatcat tgcagcagac aagatagtgg   4500
cgatagggtc aaccttattc tttggcaaat ctggagcaga accgtggcat ggttcgtaca   4560
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tctattttgt ctctatatac tacgtatagg aaatgtttac attttcgtat tgttttcgat   8760

tcactctatg aatagttctt actacaattt ttttgtctaa agagtaatac tagagataaa   8820

cataaaaaat gtagaggtcg agtttagatg caagttcaag gagcgaaagg tggatgggta   8880

ggttatatag ggatatagca cagagatata tagcaaagag atacttttga gcaatgtttg   8940

tggaagcggt attcgcaata ttttagtagc tcgttacagt ccggtgcgtt tttggttttt   9000

tgaaagtgcg tcttcagagc gcttttggtt ttcaaaagcg ctctgaagtt cctatacttt   9060
```

```
ctagagaata ggaacttcgg aataggaact tcaaagcgtt tccgaaaacg agcgcttccg   9120
aaaatgcaac gcgagctgcg cacatacagc tcactgttca cgtcgcacct atatctgcgt   9180
gttgcctgta tatatatata catgagaaga acggcatagt gcgtgtttat gcttaaatgc   9240
gtacttatat gcgtctattt atgtaggatg aaaggtagtc tagtacctcc tgtgatatta   9300
tcccattcca tgcggggtat cgtatgcttc cttcagcact accctttagc tgttctatat   9360
gctgccactc ctcaattgga ttagtctcat ccttcaatgc tatcatttcc tttgatattg   9420
gatcatctaa gaaaccatta ttatcatgac attaacctat aaaaataggc gtatcacgag   9480
gccctttcgt c                                                        9491
```

<210> SEQ ID NO 54
<211> LENGTH: 1000
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FBA1 terminator nt 4861 to 5860

<400> SEQUENCE: 54

```
gttaattcaa attaattgat atagtttttt aatgagtatt gaatctgttt agaaataatg     60
gaatattatt tttatttatt tatttatatt attggtcggc tcttttcttc tgaaggtcaa    120
tgacaaaatg atatgaagga aataatgatt tctaaaattt tacaacgtaa gatattttta    180
caaaagccta gctcatcttt tgtcatgcac tattttactc acgcttgaaa ttaacggcca    240
gtccactgcg gagtcatttc aaagtcatcc taatcgatct atcgtttttg atagctcatt    300
ttggagttcg cgattgtctt ctgttattca caactgtttt aatttttatt tcattctgga    360
actcttcgag ttctttgtaa agtctttcat agtagcttac tttatcctcc aacatattta    420
acttcatgtc aatttcggct cttaaatttt ccacatcatc aagttcaaca tcatctttta    480
acttgaattt attctctagc tcttccaacc aagcctcatt gctccttgat ttactggtga    540
aaagtgatac actttgcgcg caatccaggt caaaactttc ctgcaaagaa ttcaccaatt    600
tctcgacatc atagtacaat ttgttttgtt ctcccatcac aatttaatat acctgatgga    660
ttcttatgaa gcgctgggta atggacgtgt cactctactt cgcctttttc cctactcctt    720
ttagtacgga agacaatgct aataaataag agggtaataa taatattatt aatcggcaaa    780
aaagattaaa cgccaagcgt ttaattatca gaaagcaaac gtcgtaccaa tccttgaatg    840
cttcccaatt gtatattaag agtcatcaca gcaacatatt cttgttatta aattaattat    900
tattgatttt tgatattgta taaaaaaacc aaatatgtat aaaaaaagtg aataaaaaat    960
accaagtatg gagaaatata ttagaagtct atacgttaaa                         1000
```

<210> SEQ ID NO 55
<211> LENGTH: 1713
<212> TYPE: DNA
<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 55

```
atgactgaca aaaaaactct taaagactta agaaatcgta gttctgttta cgattcaatg     60
gttaaatcac ctaatcgtgc tatgttgcgt gcaactggta tgcaagatga agactttgaa    120
aaacctatcg tcggtgtcat ttcaacttgg gctgaaaaca caccttgtaa tatccactta    180
catgactttg gtaaactagc caaagtcggt gttaaggaag ctggtgcttg gccagttcag    240
ttcggaacaa tcacggtttc tgatggaatc gccatgggaa cccaaggaat gcgtttctcc    300
```

```
ttgacatctc gtgatattat tgcagattct attgaagcag ccatgggagg tcataatgcg    360

gatgcttttg tagccattgg cggttgtgat aaaaacatgc ccggttctgt tatcgctatg    420

gctaacatgg atatcccagc catttttgct tacggcggaa caattgcacc tggtaattta    480

gacggcaaag atatcgattt agtctctgtc tttgaaggtg tcggccattg gaaccacggc    540

gatatgacca aagaagaagt taaagctttg gaatgtaatg cttgtcccgg tcctggaggc    600

tgcggtggta tgtatactgc taacacaatg gcgacagcta ttgaagtttt gggacttagc    660

cttccgggtt catcttctca cccggctgaa tccgcagaaa agaaagcaga tattgaagaa    720

gctggtcgcg ctgttgtcaa aatgctcgaa atgggcttaa aaccttctga cattttaacg    780

cgtgaagctt ttgaagatgc tattactgta actatggctc tgggaggttc aaccaactca    840

acccttcacc tcttagctat tgcccatgct gctaatgtgg aattgacact tgatgatttc    900

aatactttcc aagaaaaagt tcctcatttg gctgatttga aaccttctgg tcaatatgta    960

ttccaagacc tttacaaggt cggaggggta ccagcagtta tgaaatatct ccttaaaaat   1020

ggcttccttc atggtgaccg tatcacttgt actggcaaaa cagtcgctga aaatttgaag   1080

gcttttgatg atttaacacc tggtcaaaag gttattatgc cgcttgaaaa tcctaaacgt   1140

gaagatggtc cgctcattat tctccatggt aacttggctc cagacggtgc cgttgccaaa   1200

gtttctggtg taaaagtgcg tcgtcatgtc ggtcctgcta aggtctttaa ttctgaagaa   1260

gaagccattg aagctgtctt gaatgatgat attgttgatg gtgatgttgt tgtcgtacgt   1320

tttgtaggac caaagggcgg tcctggtatg cctgaaatgc tttccctttc atcaatgatt   1380

gttggtaaag ggcaaggtga aaaagttgcc cttctgacag atggccgctt ctcaggtggt   1440

acttatggtc ttgtcgtggg tcatatcgct cctgaagcac aagatggcgg tccaatcgcc   1500

tacctgcaaa caggagacat agtcactatt gaccaagaca ctaaggaatt acactttgat   1560

atctccgatg aagagttaaa acatcgtcaa gagaccattg aattgccacc gctctattca   1620

cgcggtatcc ttggtaaata tgctcacatc gtttcgtctg cttctagggg agccgtaaca   1680

gacttttgga agcctgaaga aactggcaaa aaa                                1713


<210> SEQ ID NO 56
<211> LENGTH: 571
<212> TYPE: PRT
<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 56

Met Thr Asp Lys Lys Thr Leu Lys Asp Leu Arg Asn Arg Ser Ser Val
1               5                   10                  15

Tyr Asp Ser Met Val Lys Ser Pro Asn Arg Ala Met Leu Arg Ala Thr
            20                  25                  30

Gly Met Gln Asp Glu Asp Phe Glu Lys Pro Ile Val Gly Val Ile Ser
        35                  40                  45

Thr Trp Ala Glu Asn Thr Pro Cys Asn Ile His Leu His Asp Phe Gly
    50                  55                  60

Lys Leu Ala Lys Val Gly Val Lys Glu Ala Gly Ala Trp Pro Val Gln
65                  70                  75                  80

Phe Gly Thr Ile Thr Val Ser Asp Gly Ile Ala Met Gly Thr Gln Gly
                85                  90                  95

Met Arg Phe Ser Leu Thr Ser Arg Asp Ile Ile Ala Asp Ser Ile Glu
            100                 105                 110

Ala Ala Met Gly Gly His Asn Ala Asp Ala Phe Val Ala Ile Gly Gly
        115                 120                 125
```

```
Cys Asp Lys Asn Met Pro Gly Ser Val Ile Ala Met Ala Asn Met Asp
    130                 135                 140

Ile Pro Ala Ile Phe Ala Tyr Gly Gly Thr Ile Ala Pro Gly Asn Leu
145                 150                 155                 160

Asp Gly Lys Asp Ile Asp Leu Val Ser Val Phe Glu Gly Val Gly His
                165                 170                 175

Trp Asn His Gly Asp Met Thr Lys Glu Glu Val Lys Ala Leu Glu Cys
            180                 185                 190

Asn Ala Cys Pro Gly Pro Gly Gly Cys Gly Gly Met Tyr Thr Ala Asn
        195                 200                 205

Thr Met Ala Thr Ala Ile Glu Val Leu Gly Leu Ser Leu Pro Gly Ser
    210                 215                 220

Ser Ser His Pro Ala Glu Ser Ala Glu Lys Lys Ala Asp Ile Glu Glu
225                 230                 235                 240

Ala Gly Arg Ala Val Val Lys Met Leu Glu Met Gly Leu Lys Pro Ser
                245                 250                 255

Asp Ile Leu Thr Arg Glu Ala Phe Glu Asp Ala Ile Thr Val Thr Met
            260                 265                 270

Ala Leu Gly Gly Ser Thr Asn Ser Thr Leu His Leu Leu Ala Ile Ala
        275                 280                 285

His Ala Ala Asn Val Glu Leu Thr Leu Asp Asp Phe Asn Thr Phe Gln
    290                 295                 300

Glu Lys Val Pro His Leu Ala Asp Leu Lys Pro Ser Gly Gln Tyr Val
305                 310                 315                 320

Phe Gln Asp Leu Tyr Lys Val Gly Gly Val Pro Ala Val Met Lys Tyr
                325                 330                 335

Leu Leu Lys Asn Gly Phe Leu His Gly Asp Arg Ile Thr Cys Thr Gly
            340                 345                 350

Lys Thr Val Ala Glu Asn Leu Lys Ala Phe Asp Asp Leu Thr Pro Gly
        355                 360                 365

Gln Lys Val Ile Met Pro Leu Glu Asn Pro Lys Arg Glu Asp Gly Pro
    370                 375                 380

Leu Ile Ile Leu His Gly Asn Leu Ala Pro Asp Gly Ala Val Ala Lys
385                 390                 395                 400

Val Ser Gly Val Lys Val Arg Arg His Val Gly Pro Ala Lys Val Phe
                405                 410                 415

Asn Ser Glu Glu Glu Ala Ile Glu Ala Val Leu Asn Asp Asp Ile Val
            420                 425                 430

Asp Gly Asp Val Val Val Val Arg Phe Val Gly Pro Lys Gly Gly Pro
        435                 440                 445

Gly Met Pro Glu Met Leu Ser Leu Ser Ser Met Ile Val Gly Lys Gly
    450                 455                 460

Gln Gly Glu Lys Val Ala Leu Leu Thr Asp Gly Arg Phe Ser Gly Gly
465                 470                 475                 480

Thr Tyr Gly Leu Val Val Gly His Ile Ala Pro Glu Ala Gln Asp Gly
                485                 490                 495

Gly Pro Ile Ala Tyr Leu Gln Thr Gly Asp Ile Val Thr Ile Asp Gln
            500                 505                 510

Asp Thr Lys Glu Leu His Phe Asp Ile Ser Asp Glu Glu Leu Lys His
        515                 520                 525

Arg Gln Glu Thr Ile Glu Leu Pro Pro Leu Tyr Ser Arg Gly Ile Leu
    530                 535                 540
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Gly Lys Tyr Ala His Ile Val Ser Ser Ala Ser Arg Gly Ala Val Thr
545                 550                 555                 560

Asp Phe Trp Lys Pro Glu Glu Thr Gly Lys Lys
                565                 570


<210> SEQ ID NO 57
<211> LENGTH: 4280
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pUC19-URA3r

<400> SEQUENCE: 57

ggggatcctc tagagtcgac ctgcaggcat gcaagcttgg cgtaatcatg gtcatagctg     60

tttcctgtgt gaaattgtta tccgctcaca attccacaca acatacgagc cggaagcata    120

aagtgtaaag cctggggtgc ctaatgagtg agctaactca cattaattgc gttgcgctca    180

ctgcccgctt tccagtcggg aaacctgtcg tgccagctgc attaatgaat cggccaacgc    240

gcggggagag gcggtttgcg tattgggcgc tcttccgctt cctcgctcac tgactcgctg    300

cgctcggtcg ttcggctgcg gcgagcggta tcagctcact caaaggcggt aatacggtta    360

tccacagaat caggggataa cgcaggaaag aacatgtgag caaaaggcca gcaaaaggcc    420

aggaaccgta aaaaggccgc gttgctggcg tttttccata ggctccgccc ccctgacgag    480

catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact ataaagatac    540

caggcgtttc cccctggaag ctccctcgtg cgctctcctg ttccgaccct gccgcttacc    600

ggatacctgt ccgcctttct cccttcggga agcgtggcgc tttctcatag ctcacgctgt    660

aggtatctca gttcggtgta ggtcgttcgc tccaagctgg gctgtgtgca cgaacccccc    720

gttcagcccg accgctgcgc cttatccggt aactatcgtc ttgagtccaa cccggtaaga    780

cacgacttat cgccactggc agcagccact ggtaacagga ttagcagagc gaggtatgta    840

ggcggtgcta cagagttctt gaagtggtgg cctaactacg gctacactag aaggacagta    900

tttggtatct gcgctctgct gaagccagtt accttcggaa aaagagttgg tagctcttga    960

tccggcaaac aaaccaccgc tggtagcggt ggtttttttg tttgcaagca gcagattacg   1020

cgcagaaaaa aaggatctca agaagatcct ttgatctttt ctacggggtc tgacgctcag   1080

tggaacgaaa actcacgtta agggattttg gtcatgagat tatcaaaaag gatcttcacc   1140

tagatccttt taaattaaaa atgaagtttt aaatcaatct aaagtatata tgagtaaact   1200

tggtctgaca gttaccaatg cttaatcagt gaggcaccta tctcagcgat ctgtctattt   1260

cgttcatcca tagttgcctg actccccgtc gtgtagataa ctacgatacg ggagggctta   1320

ccatctggcc ccagtgctgc aatgataccg cgagacccac gctcaccggc tccagattta   1380

tcagcaataa accagccagc cggaagggcc gagcgcagaa gtggtcctgc aactttatcc   1440

gcctccatcc agtctattaa ttgttgccgg gaagctagag taagtagttc gccagttaat   1500

agtttgcgca acgttgttgc cattgctaca ggcatcgtgg tgtcacgctc gtcgtttggt   1560

atggcttcat tcagctccgg ttcccaacga tcaaggcgag ttacatgatc ccccatgttg   1620

tgcaaaaaag cggttagctc cttcggtcct ccgatcgttg tcagaagtaa gttggccgca   1680

gtgttatcac tcatggttat ggcagcactg cataattctc ttactgtcat gccatccgta   1740

agatgctttt ctgtgactgg tgagtactca accaagtcat tctgagaata gtgtatgcgg   1800

cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca tagcagaact   1860

ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag gatcttaccg   1920
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```
ctgttgagat ccagttcgat gtaacccact cgtgcaccca actgatcttc agcatctttt   1980
actttcacca gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc aaaaaaggga   2040
ataagggcga cacggaaatg ttgaatactc atactcttcc tttttcaata ttattgaagc   2100
atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta gaaaaataaa   2160
caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgtcta agaaaccatt   2220
attatcatga cattaaccta taaaaatagg cgtatcacga ggccctttcg tctcgcgcgt   2280
ttcggtgatg acggtgaaaa cctctgacac atgcagctcc cggagacggt cacagcttgt   2340
ctgtaagcgg atgccgggag cagacaagcc cgtcagggcg cgtcagcggg tgttggcggg   2400
tgtcggggct ggcttaacta tgcggcatca gagcagattg tactgagagt gcaccatatg   2460
cggtgtgaaa taccgcacag atgcgtaagg agaaaatacc gcatcaggcg ccattcgcca   2520
ttcaggctgc gcaactgttg ggaagggcga tcggtgcggg cctcttcgct attacgccag   2580
ctggcgaaag ggggatgtgc tgcaaggcga ttaagttggg taacgccagg gttttcccag   2640
tcacgacgtt gtaaaacgac ggccagtgaa ttcgagctcg gtacccccgg ctctgagaca   2700
gtagtaggtt agtcatcgct ctaccgacgc gcaggaaaag aaagaagcat tgcggattac   2760
gtattctaat gttcagcccg cggaacgcca gcaaatcacc acccatgcgc atgatactga   2820
gtcttgtaca cgctgggctt ccagtgtact gagagtgcac cataccacag cttttcaatt   2880
caattcatca tttttttttt attctttttt ttgatttcgg tttctttgaa attttttttga  2940
ttcggtaatc tccgaacaga aggaagaacg aaggaaggag cacagactta gattggtata   3000
tatacgcata tgtagtgttg aagaaacatg aaattgccca gtattcttaa cccaactgca   3060
cagaacaaaa acctgcagga aacgaagata aatcatgtcg aaagctacat ataaggaacg   3120
tgctgctact catcctagtc ctgttgctgc caagctattt aatatcatgc acgaaaagca   3180
aacaaacttg tgtgcttcat tggatgttcg taccaccaag gaattactgg agttagttga   3240
agcattaggt cccaaaattt gtttactaaa aacacatgtg gatatcttga ctgatttttc   3300
catggagggc acagttaagc cgctaaaggc attatccgcc aagtacaatt ttttactctt   3360
cgaagacaga aaatttgctg acattggtaa tacagtcaaa ttgcagtact ctgcgggtgt   3420
atacagaata gcagaatggg cagacattac gaatgcacac ggtgtggtgg gcccaggtat   3480
tgttagcggt ttgaagcagg cggcagaaga agtaacaaag gaacctagag gccttttgat   3540
gttagcagaa ttgtcatgca agggctccct atctactgga gaatatacta agggtactgt   3600
tgacattgcg aagagcgaca aagattttgt tatcggcttt attgctcaaa gagacatggg   3660
tggaagagat gaaggttacg attggttgat tatgacaccc ggtgtgggtt tagatgacaa   3720
gggagacgca ttgggtcaac agtatagaac cgtggatgat gtggtctcta caggatctga   3780
cattattatt gttggaagag gactatttgc aaagggaagg gatgctaagg tagagggtga   3840
acgttacaga aaagcaggct gggaagcata tttgagaaga tgcggccagc aaaactaaaa   3900
aactgtatta taagtaaatg catgtatact aaactcacaa attagagctt caatttaatt   3960
atatcagtta ttaccctatg cggtgtgaaa taccgcacag atgcgtaagg agaaaatacc   4020
gcatcaggaa attgtaaacg ttaatatttt gttaaaattc gcgttaaatt tttgttaaat   4080
cagctcattt tttaaccaat aggccgaaat cggcaaaatc ttcagcccgc ggaacgccag   4140
caaatcacca cccatgcgca tgatactgag tcttgtacac gctgggcttc cagtgatgat   4200
acaacgagtt agccaaggtg agcacggatg tctaaattag aattacgttt taatatcttt   4260
```

ttttccatat ctagggctag 4280

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-11A

<400> SEQUENCE: 58

gcatgcttgc atttagtcgt gcaatgtatg 30

<210> SEQ ID NO 59
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-11B

<400> SEQUENCE: 59

gaacattaga atacgtaatc cgcaatgcac tagtaccaca ggtgttgtcc tctg 54

<210> SEQ ID NO 60
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-11C

<400> SEQUENCE: 60

cagaggacaa cacctgtggt actagtgcat tgcggattac gtattctaat gttc 54

<210> SEQ ID NO 61
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-11D

<400> SEQUENCE: 61

caccttggct aactcgttgt atcatcac 28

<210> SEQ ID NO 62
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-13A

<400> SEQUENCE: 62

ttttaagccg aatgagtgac agaaaaagcc cacaacttat caagtgatat tgaacaaagg 60

gcgaaacttc gcatgcttgc atttagtcgt gcaatgtatg 100

<210> SEQ ID NO 63
<211> LENGTH: 98
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-13B

<400> SEQUENCE: 63

cccaattggt aaatattcaa caagagacgc gcagtacgta acatgcgaat tgcgtaattc 60

acggcgataa caccttggct aactcgttgt atcatcac 98

<210> SEQ ID NO 64
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 112590-34G

<400> SEQUENCE: 64

caaaagccca tgtcccacac caaaggatg 29

<210> SEQ ID NO 65
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 112590-34H

<400> SEQUENCE: 65

caccatcgcg cgtgcatcac tgcatg 26

<210> SEQ ID NO 66
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 112590-34F

<400> SEQUENCE: 66

tcggtttttg caatatgacc tgtgggcc 28

<210> SEQ ID NO 67
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 112590-49E

<400> SEQUENCE: 67

gagaagatgc ggccagcaaa ac 22

<210> SEQ ID NO 68
<211> LENGTH: 99
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-27A

<400> SEQUENCE: 68

tcctttctca attattattt tctactcata acctcacgca aaataacaca gtcaaatcaa 60

tcaaagtatg actgacaaaa aaactcttaa agacttaag 99

<210> SEQ ID NO 69
<211> LENGTH: 77
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-27B

<400> SEQUENCE: 69

gaacattaga atacgtaatc cgcaatgctt ctttcttttc cgtttaacgt atagacttct 60

aatatatttc tccatac 77

<210> SEQ ID NO 70
<211> LENGTH: 45
<212> TYPE: DNA

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-27C

<400> SEQUENCE: 70

aaacggaaaa gaaagaagca ttgcggatta cgtattctaa tgttc 45

<210> SEQ ID NO 71
<211> LENGTH: 88
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-27D

<400> SEQUENCE: 71

tatttttcgt tacataaaaa tgcttataaa actttaacta ataattagag attaaatcgc 60

caccttggct aactcgttgt atcatcac 88

<210> SEQ ID NO 72
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-36D

<400> SEQUENCE: 72

gacttttgga agcctgaaga aactggc 27

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 135

<400> SEQUENCE: 73

cttggcagca acaggactag 20

<210> SEQ ID NO 74
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 112590-30F

<400> SEQUENCE: 74

ccaggccaat tcaacagact gtcggc 26

<210> SEQ ID NO 75
<211> LENGTH: 2347
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: URA3r2 template DNA

<400> SEQUENCE: 75

gcattgcgga ttacgtattc taatgttcag gtgctggaag aagagctgct taaccgccgc 60

gcccagggtg aagatccacg ctactttacc ctgcgtcgtc tggatttcgg cggctgtcgt 120

ctttcgctgg caacgccggt tgatgaagcc tgggacggtc cgctctcctt aaacggtaaa 180

cgtatcgcca cctcttatcc tcacctgctc aagcgttatc tcgaccagaa aggcatctct 240

tttaaatcct gcttactgaa cggttctgtt gaagtcgccc cgcgtgccgg actggcggat 300

gcgatttgcg atctggtttc caccggtgcc acgctggaag ctaacggcct gcgcgaagtc 360

```
gaagttatct atcgctcgaa agcctgcctg attcaacgcg atggcgaaat ggaagaatcc    420

aaacagcaac tgatcgacaa actgctgacc cgtattcagg gtgtgatcca ggcgcgcgaa    480

tcaaaataca tcatgatgca cgcaccgacc gaacgtctgg atgaagtcat ggtacctact    540

gagagtgcac cataccacag cttttcaatt caattcatca tttttttttt attctttttt    600

ttgatttcgg tttctttgaa attttttga ttcggtaatc tccgaacaga aggaagaacg    660

aaggaaggag cacagactta gattggtata tatacgcata tgtagtgttg aagaaacatg    720

aaattgccca gtattcttaa cccaactgca cagaacaaaa acctgcagga aacgaagata    780

aatcatgtcg aaagctacat ataaggaacg tgctgctact catcctagtc ctgttgctgc    840

caagctattt aatatcatgc acgaaaagca aacaaacttg tgtgcttcat tggatgttcg    900

taccaccaag gaattactgg agttagttga agcattaggt cccaaaattt gtttactaaa    960

aacacatgtg gatatcttga ctgatttttc catggagggc acagttaagc cgctaaaggc   1020

attatccgcc aagtacaatt ttttactctt cgaagacaga aaatttgctg acattggtaa   1080

tacagtcaaa ttgcagtact ctgcgggtgt atacagaata gcagaatggg cagacattac   1140

gaatgcacac ggtgtggtgg gcccaggtat tgttagcggt ttgaagcagg cggcagaaga   1200

agtaacaaag gaacctagag gccttttgat gttagcagaa ttgtcatgca agggctccct   1260

atctactgga gaatatacta agggtactgt tgacattgcg aagagcgaca aagattttgt   1320

tatcggcttt attgctcaaa gagacatggg tggaagagat gaaggttacg attggttgat   1380

tatgacaccc ggtgtgggtt tagatgacaa gggagacgca ttgggtcaac agtatagaac   1440

cgtggatgat gtggtctcta caggatctga cattattatt gttggaagag gactatttgc   1500

aaagggaagg gatgctaagg tagagggtga acgttacaga aaagcaggct gggaagcata   1560

tttgagaaga tgcggccagc aaaactaaaa aactgtatta taagtaaatg catgtatact   1620

aaactcacaa attagagctt caatttaatt atatcagtta ttaccctatg cggtgtgaaa   1680

taccgcacag atgcgtaagg agaaaatacc gcatcaggaa attgtaaacg ttaatatttt   1740

gttaaaattc gcgttaaatt tttgttaaat cagctcattt tttaaccaat aggccgaaat   1800

cggcaaaatc tctagagtgc tggaagaaga gctgcttaac cgccgcgccc agggtgaaga   1860

tccacgctac tttaccctgc gtcgtctgga tttcggcggc tgtcgtcttt cgctggcaac   1920

gccggttgat gaagcctggg acggtccgct ctccttaaac ggtaaacgta tcgccacctc   1980

ttatcctcac ctgctcaagc gttatctcga ccagaaaggc atctctttta aatcctgctt   2040

actgaacggt tctgttgaag tcgccccgcg tgccggactg gcggatgcga tttgcgatct   2100

ggtttccacc ggtgccacgc tggaagctaa cggcctgcgc gaagtcgaag ttatctatcg   2160

ctcgaaagcc tgcctgattc aacgcgatgg cgaaatggaa gaatccaaac agcaactgat   2220

cgacaaactg ctgacccgta ttcagggtgt gatccaggcg cgcgaatcaa aatacatcat   2280

gatgcacgca ccgaccgaac gtctggatga agtcatccag tgatgataca acgagttagc   2340

caaggtg                                                             2347
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<210> SEQ ID NO 76
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-45A

<400> SEQUENCE: 76

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cttcgaagaa tatactaaaa aatgagcagg caagataaac gaaggcaaag gcattgcgga     60

ttacgtattc taatgttcag                                                 80


<210> SEQ ID NO 77
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 114117-45B

<400> SEQUENCE: 77

tatacacatg tatatatatc gtatgctgca gctttaaata atcggtgtca caccttggct     60

aactcgttgt atcatcactg g                                               81


<210> SEQ ID NO 78
<211> LENGTH: 90
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 384

<400> SEQUENCE: 78

atggttcatt taggtccaaa aaaaccacaa gccagaaagg gttccatggc cgatgtgcca     60

gcattgcgga ttacgtattc taatgttcag                                      90


<210> SEQ ID NO 79
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer 385

<400> SEQUENCE: 79

ttaagcaccg atgataccaa cggacttacc ttcagcaatt ctttttttggg ccaaagcagc     60

caccttggct aactcgttgt atcatcactg g                                    91


<210> SEQ ID NO 80
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer N869

<400> SEQUENCE: 80

ctaggatgag tagcagcacg ttcc                                            24


<210> SEQ ID NO 81
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer N871

<400> SEQUENCE: 81

ccaattccgt gatgtctctt tgttgc                                          26


<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer N946

<400> SEQUENCE: 82
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gtgaacgagt tcacaaccgc 20

<210> SEQ ID NO 83
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer N947

<400> SEQUENCE: 83

gttcgttcca gaattatcac gc 22

<210> SEQ ID NO 84
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer PDC5:KanMXF

<400> SEQUENCE: 84

gacttgaata atgcagcggc gcttgc 26

<210> SEQ ID NO 85
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer PDC5::KanMXR

<400> SEQUENCE: 85

ccaccctctt caattagcta agatcatagc 30

<210> SEQ ID NO 86
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP457

<400> SEQUENCE: 86

ccagaaaccc tatacctgtg tggacgtaag gccatgaagc tttttcttt 49

<210> SEQ ID NO 87
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP458

<400> SEQUENCE: 87

attggaaaga aaaagcttca tggccttacg tccacacagg tatagggtt 49

<210> SEQ ID NO 88
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP459

<400> SEQUENCE: 88

cataagaaca cctttggtgg ag 22

<210> SEQ ID NO 89
<211> LENGTH: 24
<212> TYPE: DNA

<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP452

<400> SEQUENCE: 89

ttctcgacgt gggccttttt cttg 24

<210> SEQ ID NO 90
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP455

<400> SEQUENCE: 90

tatggaccct gaaaccacag ccacattgta accaccacga cggttgttg 49

<210> SEQ ID NO 91
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer oBP456

<400> SEQUENCE: 91

tttagcaaca accgtcgtgg tggttacaat gtggctgtgg tttcagggt 49

<210> SEQ ID NO 92
<211> LENGTH: 512
<212> TYPE: PRT
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 92

Met Ala Leu Leu Ala Val Ala Gly Val Tyr Ala Phe Ala Ala Leu Leu
1 5 10 15

Val Ala Ile Val Leu Asn Val Thr Arg Gln Leu Leu Phe Arg Asn Glu
20 25 30

Lys Glu Pro Pro Val Val Phe His Trp Ile Pro Phe Leu Gly Ser Thr
35 40 45

Ile Ser Tyr Gly Met Asp Pro Tyr Thr Phe Phe Phe Ser Cys Arg Lys
50 55 60

Lys Tyr Gly Asp Ile Phe Thr Phe Val Leu Leu Gly Gln Lys Thr Thr
65 70 75 80

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

Lys Asp Val Ser Ala Glu Glu Val Tyr Ser Pro Leu Thr Thr Pro Val
100 105 110

Phe Gly Ser Asp Val Val Tyr Asp Cys Pro Asn Ser Lys Leu Met Glu
115 120 125

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

His Val Gln Leu Ile Glu Lys Glu Thr Leu Asp Tyr Leu Arg Asp Ser
145 150 155 160

Pro Arg Phe Asn Gly Ala Ser Gly Val Ile Asp Ile Pro Ala Ala Met
165 170 175

Ala Glu Ile Thr Ile Tyr Thr Ala Ala Arg Ala Leu Gln Gly Glu Glu
180 185 190

Val Arg Lys Lys Leu Thr Ala Glu Phe Ala Glu Leu Tyr His Asp Leu
195 200 205

```
Asp Lys Gly Phe Ser Pro Ile Asn Phe Met Leu Pro Trp Ala Pro Leu
    210                 215                 220

Pro His Asn Arg Lys Arg Asp Ala Ala His Ala Arg Met Arg Glu Ile
225                 230                 235                 240

Tyr Thr Asp Ile Ile Asn Glu Arg Arg Lys Asn Pro Asp Glu Glu Lys
                245                 250                 255

Ser Asp Met Ile Trp Asn Leu Met His Cys Thr Tyr Lys Ser Gly Gln
            260                 265                 270

Pro Val Pro Asp Lys Glu Ile Ala His Met Met Ile Thr Leu Leu Met
        275                 280                 285

Ala Gly Gln His Ser Ser Ser Ser Ile Ser Ser Trp Ile Met Leu Arg
    290                 295                 300

Leu Ala Ser Glu Pro Gln Val Leu Glu Glu Leu Tyr Gln Glu Gln Leu
305                 310                 315                 320

Ala Ser Leu Ser Asn Arg Asn Gly Val Phe Glu Pro Leu Gln Tyr Gln
                325                 330                 335

Asp Leu Asp Lys Leu Pro Phe Leu Gln Ser Val Ile Lys Glu Thr Leu
            340                 345                 350

Arg Ile His Ser Ser Ile His Ser Ile Met Arg Lys Val Lys Asn Pro
        355                 360                 365

Leu Pro Val Pro Gly Thr Ser Tyr Ile Ile Pro Glu Asp His Val Leu
    370                 375                 380

Leu Ala Ser Pro Gly Val Thr Ala Leu Ser Asp Glu Tyr Phe Pro Asn
385                 390                 395                 400

Ala Thr Arg Trp Asp Pro His Arg Trp Glu Asn Gln Pro Asp Lys Glu
                405                 410                 415

Glu Asp Gly Glu Met Val Asp Tyr Gly Tyr Gly Ser Val Ser Lys Gly
            420                 425                 430

Thr Ala Ser Pro Tyr Leu Pro Phe Gly Ala Gly Arg His Arg Cys Ile
        435                 440                 445

Gly Glu Lys Phe Ala Tyr Val Asn Leu Gly Val Ile Ile Ala Thr Ile
    450                 455                 460

Val Arg His Leu Lys Leu Phe Asn Val Asp Gly Arg Lys Gly Val Pro
465                 470                 475                 480

Gly Thr Asp Tyr Ser Thr Leu Phe Ser Gly Pro Met Lys Pro Ala Ile
                485                 490                 495

Val Gly Trp Glu Arg Arg Phe Pro Asp Asn Ile Lys Gly Ser Met Asn
            500                 505                 510
```

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<210> SEQ ID NO 93
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP460

<400> SEQUENCE: 93

aggattatca ttcataagtt tc                                              22


<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA135

<400> SEQUENCE: 94
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cttggcagca acaggactag                                              20


<210> SEQ ID NO 95
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer BP461

<400> SEQUENCE: 95

ttcttggagc tgggacatgt ttg                                          23


<210> SEQ ID NO 96
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA92

<400> SEQUENCE: 96

gagaagatgc ggccagcaaa ac                                           22


<210> SEQ ID NO 97
<211> LENGTH: 4242
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: pLA59

<400> SEQUENCE: 97

aaacgccagc aacgcggcct ttttacggtt cctggccttt tgctggcctt ttgctcacat     60

gttctttcct gcgttatccc ctgattctgt ggataaccgt attaccgcct ttgagtgagc    120

tgataccgct cgccgcagcc gaacgaccga gcgcagcgag tcagtgagcg aggaagcgga    180

agagcgccca atacgcaaac cgcctctccc cgcgcgttgg ccgattcatt aatgcagctg    240

gcacgacagg tttcccgact ggaaagcggg cagtgagcgc aacgcaatta atgtgagtta    300

gctcactcat taggcacccc aggctttaca ctttatgctt ccggctcgta tgttgtgtgg    360

aattgtgagc ggataacaat ttcacacagg aaacagctat gaccatgatt acgccaagct    420

tgcatgcctg caggtcgact ctagaggatc cgcaatgcgg atccgcattg cggattacgt    480

attctaatgt tcagtaccgt tcgtataatg tatgctatac gaagttatgc agattgtact    540

gagagtgcac cataccacct tttcaattca tcattttttt tttattcttt tttttgattt    600

cggtttcctt gaaatttttt tgattcggta atctccgaac agaaggaaga acgaaggaag    660

gagcacagac ttagattggt atatatacgc atatgtagtg ttgaagaaac atgaaattgc    720

ccagtattct taacccaact gcacagaaca aaaacctgca ggaaacgaag ataaatcatg    780

tcgaaagcta catataagga acgtgctgct actcatccta gtcctgttgc tgccaagcta    840

tttaatatca tgcacgaaaa gcaaacaaac ttgtgtgctt cattggatgt tcgtaccacc    900

aaggaattac tggagttagt tgaagcatta ggtcccaaaa tttgtttact aaaaacacat    960

gtggatatct tgactgattt ttccatggag ggcacagtta agccgctaaa ggcattatcc   1020

gccaagtaca atttttttact cttcgaagac agaaaatttg ctgacattgg taatacagtc   1080

aaattgcagt actctgcggg tgtatacaga atagcagaat gggcagacat tacgaatgca   1140

cacggtgtgg tgggcccagg tattgttagc ggtttgaagc aggcggcaga agaagtaaca   1200

aaggaaccta gaggcctttt gatgttagca gaattgtcat gcaagggctc cctatctact   1260

ggagaatata ctaagggtac tgttgacatt gcgaagagcg acaaagattt tgttatcggc   1320
```

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tttattgctc aaagagacat gggtggaaga gatgaaggtt acgattggtt gattatgaca   1380
cccggtgtgg gtttagatga caagggagac gcattgggtc aacagtatag aaccgtggat   1440
gatgtggtct ctacaggatc tgacattatt attgttggaa gaggactatt tgcaaaggga   1500
agggatgcta aggtagaggg tgaacgttac agaaaagcag gctgggaagc atatttgaga   1560
agatgcggcc agcaaaacta aaaaactgta ttataagtaa atgcatgtat actaaactca   1620
caaattagag cttcaattta attatatcag ttattaccct atgcggtgtg aaataccgca   1680
cagatgcgta aggagaaaat accgcatcag gaaattgtaa acgttaatat tttgttaaaa   1740
ttcgcgttaa atttttgtta aatcagctca ttttttaacc aataggccga aatcggcaaa   1800
atcccttata aatcaaaaga atagaccgag atagggttga gtgttgttcc agtttggaac   1860
aagagtccac tattaaagaa cgtggactcc aacgtcaaag ggcgaaaaac cgtctatcag   1920
ggcgatggcc cactacgtga accatcaccc taatcaagat aacttcgtat aatgtatgct   1980
atacgaacgg taccagtgat gatacaacga gttagccaag gtgaattcac tggccgtcgt   2040
tttacaacgt cgtgactggg aaaaccctgg cgttacccaa cttaatcgcc ttgcagcaca   2100
tccccctttc gccagctggc gtaatagcga agaggcccgc accgatcgcc cttcccaaca   2160
gttgcgcagc ctgaatggcg aatggcgcct gatgcggtat tttctcctta cgcatctgtg   2220
cggtatttca caccgcatat ggtgcactct cagtacaatc tgctctgatg ccgcatagtt   2280
aagccagccc cgacacccgc caacacccgc tgacgcgccc tgacgggctt gtctgctccc   2340
ggcatccgct tacagacaag ctgtgaccgt ctccgggagc tgcatgtgtc agaggttttc   2400
accgtcatca ccgaaacgcg cgagacgaaa gggcctcgtg atacgcctat ttttataggt   2460
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cggaacccct atttgtttat ttttctaaat acattcaaat atgtatccgc tcatgagaca   2580
ataaccctga taaatgcttc aataatattg aaaaaggaag agtatgagta ttcaacattt   2640
ccgtgtcgcc cttattccct tttttgcggc attttgcctt cctgtttttg ctcacccaga   2700
aacgctggtg aaagtaaaag atgctgaaga tcagttgggt gcacgagtgg gttacatcga   2760
actggatctc aacagcggta agatccttga gagttttcgc cccgaagaac gttttccaat   2820
gatgagcact tttaaagttc tgctatgtgg cgcggtatta tcccgtattg acgccgggca   2880
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cacagaaaag catcttacgg atggcatgac agtaagagaa ttatgcagtg ctgccataac   3000
catgagtgat aacactgcgg ccaacttact tctgacaacg atcggaggac cgaaggagct   3060
aaccgctttt ttgcacaaca tgggggatca tgtaactcgc cttgatcgtt gggaaccgga   3120
gctgaatgaa gccataccaa acgacgagcg tgacaccacg atgcctgtag caatggcaac   3180
aacgttgcgc aaactattaa ctggcgaact acttactcta gcttcccggc aacaattaat   3240
agactggatg gaggcggata aagttgcagg accacttctg cgctcggccc ttccggctgg   3300
ctggtttatt gctgataaat ctggagccgg tgagcgtggg tctcgcggta tcattgcagc   3360
actggggcca gatggtaagc cctcccgtat cgtagttatc tacacgacgg ggagtcaggc   3420
aactatggat gaacgaaata gacagatcgc tgagataggt gcctcactga ttaagcattg   3480
gtaactgtca gaccaagttt actcatatat actttagatt gatttaaaac ttcattttta   3540
atttaaaagg atctaggtga agatcctttt tgataatctc atgaccaaaa tcccttaacg   3600
tgagttttcg ttccactgag cgtcagaccc cgtagaaaag atcaaaggat cttcttgaga   3660
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tcctttttt ctgcgcgtaa tctgctgctt gcaaacaaaa aaaccaccgc taccagcggt 3720

ggtttgtttg ccggatcaag agctaccaac tctttttccg aaggtaactg gcttcagcag 3780

agcgcagata ccaaatactg tccttctagt gtagccgtag ttaggccacc acttcaagaa 3840

ctctgtagca ccgcctacat acctcgctct gctaatcctg ttaccagtgg ctgctgccag 3900

tggcgataag tcgtgtctta ccgggttgga ctcaagacga tagttaccgg ataaggcgca 3960

gcggtcgggc tgaacggggg gttcgtgcac acagcccagc ttggagcgaa cgacctacac 4020

cgaactgaga tacctacagc gtgagctatg agaaagcgcc acgcttcccg aagggagaaa 4080

ggcggacagg tatccggtaa gcggcagggt cggaacagga gagcgcacga gggagcttcc 4140

agggggaaac gcctggtatc tttatagtcc tgtcgggttt cgccacctct gacttgagcg 4200

tcgatttttg tgatgctcgt caggggggcg gagcctatgg aa 4242

<210> SEQ ID NO 98
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: LA678

<400> SEQUENCE: 98

caacgttaac accgttttcg gtttgccagg tgacttcaac ttgtccttgt gcattgcgga 60

ttacgtattc taatgttcag 80

<210> SEQ ID NO 99
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: LA679

<400> SEQUENCE: 99

gtggagcatc gaagactggc aacatgattt caatcattct gatcttagag caccttggct 60

aactcgttgt atcatcactg g 81

<210> SEQ ID NO 100
<211> LENGTH: 4586
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLA54 template DNA

<400> SEQUENCE: 100

gggtaccgag ctcgaattca ctggccgtcg ttttacaacg tcgtgactgg gaaaaccctg 60

gcgttaccca acttaatcgc cttgcagcac atcccccttt cgccagctgg cgtaatagcg 120

aagaggcccg caccgatcgc ccttcccaac agttgcgcag cctgaatggc gaatggcgcc 180

tgatgcggta ttttctcctt acgcatctgt gcggtatttc acaccgcata tggtgcactc 240

tcagtacaat ctgctctgat gccgcatagt taagccagcc ccgacacccg ccaacacccg 300

ctgacgcgcc ctgacgggct tgtctgctcc cggcatccgc ttacagacaa gctgtgaccg 360

tctccgggag ctgcatgtgt cagaggtttt caccgtcatc accgaaacgc gcgagacgaa 420

agggcctcgt gatacgccta tttttatagg ttaatgtcat gataataatg gtttcttaga 480

cgtcaggtgg cacttttcgg ggaaatgtgc gcggaacccc tatttgttta tttttctaaa 540

tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt caataatatt 600

gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc ttttttgcgg 660

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cattttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa gatgctgaag    720
atcagttggg tgcacgagtg ggttacatcg aactggatct caacagcggt aagatccttg    780
agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt ctgctatgtg    840
gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc atacactatt    900
ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga    960
cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg gccaacttac   1020
ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac atgggggatc   1080
atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca aacgacgagc   1140
gtgacaccac gatgcctgta gcaatggcaa caacgttgcg caaactatta actggcgaac   1200
tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat aaagttgcag   1260
gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa tctggagccg   1320
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ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt tactcatata   1500
tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg aagatccttt   1560
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ccgtagaaaa gatcaaagga tcttcttgag atcctttttt tctgcgcgta atctgctgct   1680
tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgttt gccggatcaa gagctaccaa   1740
ctctttttcc gaaggtaact ggcttcagca gagcgcagat accaaatact gtccttctag   1800
tgtagccgta gttaggccac cacttcaaga actctgtagc accgcctaca tacctcgctc   1860
tgctaatcct gttaccagtg gctgctgcca gtggcgataa gtcgtgtctt accgggttgg   1920
actcaagacg atagttaccg gataaggcgc agcggtcggg ctgaacgggg ggttcgtgca   1980
cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag cgtgagctat   2040
gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta agcggcaggg   2100
tcggaacagg agagcgcacg agggagcttc caggggggaaa cgcctggtat ctttatagtc   2160
ctgtcgggtt tcgccacctc tgacttgagc gtcgattttt gtgatgctcg tcaggggggc   2220
ggagcctatg gaaaaacgcc agcaacgcgg cctttttacg gttcctggcc ttttgctggc   2280
cttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg   2340
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gcgaggaagc ggaagagcgc ccaatacgca aaccgcctct ccccgcgcgt tggccgattc   2460
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ttaatgtgag ttagctcact cattaggcac cccaggcttt acactttatg cttccggctc   2580
gtatgttgtg tggaattgtg agcggataac aatttcacac aggaaacagc tatgaccatg   2640
attacgccaa gcttgcatgc ctgcaggtcg actctagagg atccccgcat tgcggattac   2700
gtattctaat gttcagataa cttcgtatag catacattat acgaagttat ctagggattc   2760
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gtgttggaca gacaactata ccagcatgga tctcttgtat cggttctttt ctcccgctct   2940
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```

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tatacagggt ttatacggtg attcctacgg caaaaatttt tcatttctaa aaaaaaaaag   3060

aaaaattttt ctttccaacg ctagaaggaa aagaaaaatc taattaaatt gatttggtga   3120

ttttctgaga gttccctttt tcatatatcg aattttgaat ataaaaggag atcgaaaaaa   3180

tttttctatt caatctgttt tctggtttta tttgatagtt tttttgtgta ttattattat   3240

ggattagtac tggtttatat gggtttttct gtataacttc tttttatttt agtttgttta   3300

atcttatttt gagttacatt atagttccct aactgcaaga gaagtaacat taaaactcga   3360

gatgggtaag gaaaagactc acgtttcgag gccgcgatta aattccaaca tggatgctga   3420

tttatatggg tataaatggg ctcgcgataa tgtcgggcaa tcaggtgcga caatctatcg   3480

attgtatggg aagcccgatg cgccagagtt gtttctgaaa catggcaaag gtagcgttgc   3540

caatgatgtt acagatgaga tggtcagact aaactggctg acggaattta tgcctcttcc   3600

gaccatcaag cattttatcc gtactcctga tgatgcatgg ttactcacca ctgcgatccc   3660

cggcaaaaca gcattccagg tattagaaga atatcctgat tcaggtgaaa atattgttga   3720

tgcgctggca gtgttcctgc gccggttgca ttcgattcct gtttgtaatt gtccttttaa   3780

cagcgatcgc gtatttcgtc tcgctcaggc gcaatcacga atgaataacg gtttggttga   3840

tgcgagtgat tttgatgacg agcgtaatgg ctggcctgtt gaacaagtct ggaaagaaat   3900

gcataagctt ttgccattct caccggattc agtcgtcact catggtgatt tctcacttga   3960

taaccttatt tttgacgagg ggaaattaat aggttgtatt gatgttggac gagtcggaat   4020

cgcagaccga taccaggatc ttgccatcct atggaactgc ctcggtgagt tttctccttc   4080

attacagaaa cggctttttc aaaaatatgg tattgataat cctgatatga ataaattgca   4140

gtttcatttg atgctcgatg agtttttcta agtttaactt gatactacta gatttttctc   4200

cttcatttat aaaatttttg gttataattg aagctttaga agtatgaaaa aatccttttt   4260

tttcattctt tgcaaccaaa ataagaagct tcttttattc attgaaatga tgaatataaa   4320

cctaacaaaa gaaaaagact cgaatatcaa acattaaaaa aaaataaaag aggttatctg   4380

ttttcccatt tagttggagt ttgcattttc taatagatag aactctcaat taatgtggat   4440

ttagtttctc tgttcgtttt tttttgtttt gttctcactg tatttacatt tctatttagt   4500

atttagttat tcatataatc tataacttcg tatagcatac attatacgaa gttatccagt   4560

gatgatacaa cgagttagcc aaggtg                                        4586
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<210> SEQ ID NO 101
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BK505 primer

<400> SEQUENCE: 101

ttccggtttc tttgaaattt ttttgattcg gtaatctccg agcagaagga gcattgcgga     60

ttacgtattc taatgttcag                                                 80


<210> SEQ ID NO 102
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: BK506 primer

<400> SEQUENCE: 102

gggtaataac tgatataatt aaattgaagc tctaatttgt gagtttagta caccttggct     60
```

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aactcgttgt atcatcactg g                                               81


<210> SEQ ID NO 103
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: BK468 Primer

<400> SEQUENCE: 103

gcctcgagtt ttaatgttac ttctcttgca gttaggga                             38


<210> SEQ ID NO 104
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: LA492 primer

<400> SEQUENCE: 104

gctaaattcg agtgaaacac aggaagacca g                                    31


<210> SEQ ID NO 105
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AK109-1

<400> SEQUENCE: 105

agtcacatca agatcgttta tgg                                             23


<210> SEQ ID NO 106
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AK109-2

<400> SEQUENCE: 106

gcacggaata tgggactact tcg                                             23


<210> SEQ ID NO 107
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AK109-3

<400> SEQUENCE: 107

actccacttc aagtaagagt ttg                                             23


<210> SEQ ID NO 108
<211> LENGTH: 548
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 108

Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1               5                   10                  15

Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
            20                  25                  30

Asp Gln Ile Ile Ser His Lys Asp Met Lys Trp Val Gly Asn Ala Asn
        35                  40                  45
```

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Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
    50                  55                  60

Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Val
65                  70                  75                  80

Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
                85                  90                  95

Val Gly Ser Pro Thr Ser Lys Val Gln Asn Glu Gly Lys Phe Val His
            100                 105                 110

His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
        115                 120                 125

Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Val
    130                 135                 140

Glu Ile Asp Arg Val Leu Ser Ala Leu Leu Lys Glu Arg Lys Pro Val
145                 150                 155                 160

Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
                165                 170                 175

Ser Leu Pro Leu Lys Lys Glu Asn Ser Thr Ser Asn Thr Ser Asp Gln
            180                 185                 190

Glu Ile Leu Asn Lys Ile Gln Glu Ser Leu Lys Asn Ala Lys Lys Pro
        195                 200                 205

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

Val Thr Gln Phe Ile Ser Lys Thr Lys Leu Pro Ile Thr Thr Leu Asn
225                 230                 235                 240

Phe Gly Lys Ser Ser Val Asp Glu Ala Leu Pro Ser Phe Leu Gly Ile
                245                 250                 255

Tyr Asn Gly Thr Leu Ser Glu Pro Asn Leu Lys Glu Phe Val Glu Ser
            260                 265                 270

Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
        275                 280                 285

Gly Ala Phe Thr His His Leu Asn Glu Asn Lys Met Ile Ser Leu Asn
    290                 295                 300

Ile Asp Glu Gly Lys Ile Phe Asn Glu Arg Ile Gln Asn Phe Asp Phe
305                 310                 315                 320

Glu Ser Leu Ile Ser Ser Leu Leu Asp Leu Ser Glu Ile Glu Tyr Lys
                325                 330                 335

Gly Lys Tyr Ile Asp Lys Lys Gln Glu Asp Phe Val Pro Ser Asn Ala
            340                 345                 350

Leu Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Asn Leu Thr Gln
        355                 360                 365

Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
    370                 375                 380

Ser Ser Ile Phe Leu Lys Ser Lys Ser His Phe Ile Gly Gln Pro Leu
385                 390                 395                 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
                405                 410                 415

Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
            420                 425                 430

Gln Leu Thr Val Gln Glu Leu Gly Leu Ala Ile Arg Glu Lys Ile Asn
        435                 440                 445

Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
    450                 455                 460
```

```
Ile His Gly Pro Asn Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465                 470                 475                 480

Ser Lys Leu Pro Glu Ser Phe Gly Ala Thr Glu Asp Arg Val Val Ser
                485                 490                 495

Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
            500                 505                 510

Gln Ala Asp Pro Asn Arg Met Tyr Trp Ile Glu Leu Ile Leu Ala Lys
        515                 520                 525

Glu Gly Ala Pro Lys Val Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
    530                 535                 540

Gln Asn Lys Ser
545


&lt;210&gt; SEQ ID NO 109
&lt;211&gt; LENGTH: 49
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: Primer oBP453

&lt;400&gt; SEQUENCE: 109

tgcagcttta aataatcggt gtcactactt tgccttcgtt tatcttgcc                49


&lt;210&gt; SEQ ID NO 110
&lt;211&gt; LENGTH: 49
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: Primer oBP454

&lt;400&gt; SEQUENCE: 110

gagcaggcaa gataaacgaa ggcaaagtag tgacaccgat tatttaaag                49


&lt;210&gt; SEQ ID NO 111
&lt;211&gt; LENGTH: 23
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: Primer LA337

&lt;400&gt; SEQUENCE: 111

ctcatttgaa tcagcttatg gtg                                            23


&lt;210&gt; SEQ ID NO 112
&lt;211&gt; LENGTH: 24
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: LA692

&lt;400&gt; SEQUENCE: 112

ggaagtcatt gacaccatct tggc                                           24


&lt;210&gt; SEQ ID NO 113
&lt;211&gt; LENGTH: 24
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: Primer LA693

&lt;400&gt; SEQUENCE: 113

agaagctggg acagcagcgt tagc                                           24
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<210> SEQ ID NO 114
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA684

<400> SEQUENCE: 114

aatcactgca tgccttccaa aacacgaaca aggtgccggt c 41

<210> SEQ ID NO 115
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA685

<400> SEQUENCE: 115

ttaagtagga tcccacttga attgaactta ttattcatct atgac 45

<210> SEQ ID NO 116
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA682

<400> SEQUENCE: 116

gtctttacag ggcaagtctc aactagtgct atcggtac 38

<210> SEQ ID NO 117
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer LA683

<400> SEQUENCE: 117

gtaccgatag cactagttga gacttgccct gtaaagac 38

<210> SEQ ID NO 118
<211> LENGTH: 546
<212> TYPE: PRT
<213> ORGANISM: Macrococcus caseolyticus

<400> SEQUENCE: 118

Met Lys Gln Arg Ile Gly Gln Tyr Leu Ile Asp Ala Leu His Val Asn
1 5 10 15

Gly Val Asp Lys Ile Phe Gly Val Pro Gly Asp Phe Thr Leu Ala Phe
20 25 30

Leu Asp Asp Ile Ile Arg His Asp Asn Val Glu Trp Val Gly Asn Thr
35 40 45

Asn Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Val Asn
50 55 60

Gly Leu Ala Ala Val Ser Thr Thr Phe Gly Val Gly Glu Leu Ser Ala
65 70 75 80

Val Asn Gly Ile Ala Gly Ser Tyr Ala Glu Arg Val Pro Val Ile Lys
85 90 95

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

His His Ser Leu Gly Glu Gly Ile Phe Asp Ser Tyr Ser Lys Met Tyr
115 120 125

```
Ala His Ile Thr Ala Thr Thr Thr Ile Leu Ser Val Asp Asn Ala Val
    130                 135                 140

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

Val His Ile His Leu Pro Ile Asp Val Ala Leu Thr Glu Ile Glu Ile
                165                 170                 175

Pro His Ala Pro Lys Val Tyr Thr His Glu Ser Gln Asn Val Asp Ala
            180                 185                 190

Tyr Ile Gln Ala Val Glu Lys Lys Leu Met Ser Ala Lys Gln Pro Val
        195                 200                 205

Ile Ile Ala Gly His Glu Ile Asn Ser Phe Lys Leu His Glu Gln Leu
    210                 215                 220

Glu Gln Phe Val Asn Gln Thr Asn Ile Pro Val Ala Gln Leu Ser Leu
225                 230                 235                 240

Gly Lys Ser Ala Phe Asn Glu Glu Asn Glu His Tyr Leu Gly Ile Tyr
                245                 250                 255

Asp Gly Lys Ile Ala Lys Glu Asn Val Arg Glu Tyr Val Asp Asn Ala
            260                 265                 270

Asp Val Ile Leu Asn Ile Gly Ala Lys Leu Thr Asp Ser Ala Thr Ala
        275                 280                 285

Gly Phe Ser Tyr Lys Phe Asp Thr Asn Asn Ile Ile Tyr Ile Asn His
    290                 295                 300

Asn Asp Phe Lys Ala Glu Asp Val Ile Ser Asp Asn Val Ser Leu Ile
305                 310                 315                 320

Asp Leu Val Asn Gly Leu Asn Ser Ile Asp Tyr Arg Asn Glu Thr His
                325                 330                 335

Tyr Pro Ser Tyr Gln Arg Ser Asp Met Lys Tyr Glu Leu Asn Asp Ala
            340                 345                 350

Pro Leu Thr Gln Ser Asn Tyr Phe Lys Met Met Asn Ala Phe Leu Glu
        355                 360                 365

Lys Asp Asp Ile Leu Leu Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
    370                 375                 380

Tyr Asp Leu Ser Leu Tyr Lys Gly Asn Gln Phe Ile Gly Gln Pro Leu
385                 390                 395                 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ser Leu Leu Gly Ser Gln Leu
                405                 410                 415

Ala Asp Met His Arg Arg Asn Ile Leu Leu Ile Gly Asp Gly Ser Leu
            420                 425                 430

Gln Leu Thr Val Gln Ala Leu Ser Thr Met Ile Arg Lys Asp Ile Lys
        435                 440                 445

Pro Ile Ile Phe Val Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Leu
    450                 455                 460

Ile His Gly Met Glu Glu Pro Tyr Asn Asp Ile Gln Met Trp Asn Tyr
465                 470                 475                 480

Lys Gln Leu Pro Glu Val Phe Gly Gly Lys Asp Thr Val Lys Val His
                485                 490                 495

Asp Ala Lys Thr Ser Asn Glu Leu Lys Thr Val Met Asp Ser Val Lys
            500                 505                 510

Ala Asp Lys Asp His Met His Phe Ile Glu Val His Met Ala Val Glu
        515                 520                 525

Asp Ala Pro Lys Lys Leu Ile Asp Ile Ala Lys Ala Phe Ser Asp Ala
    530                 535                 540

Asn Lys
```

545

<210> SEQ ID NO 119
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA686

<400> SEQUENCE: 119

cttccaaaac acgaacaagg tgccggtc 28

<210> SEQ ID NO 120
<211> LENGTH: 97
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA687

<400> SEQUENCE: 120

gttaaaacac accatttgaa tacatatgct acgtatccac tttagattta tcaaatacta 60

ccaactcacc ttggctaact cgttgtatca tcactgg 97

<210> SEQ ID NO 121
<211> LENGTH: 7555
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: pRS423::PGAL1-cre

<400> SEQUENCE: 121

tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accataaatt cccgttttaa gagcttggtg agcgctagga gtcactgcca ggtatcgttt 240

gaacacggca ttagtcaggg aagtcataac acagtccttt cccgcaattt tctttttcta 300

ttactcttgg cctcctctag tacactctat attttttat gcctcggtaa tgattttcat 360

ttttttttt cccctagcgg atgactcttt ttttttctta gcgattggca ttatcacata 420

atgaattata cattatataa agtaatgtga tttcttcgaa gaatatacta aaaaatgagc 480

aggcaagata aacgaaggca aagatgacag agcagaaagc cctagtaaag cgtattacaa 540

atgaaaccaa gattcagatt gcgatctctt taaagggtgg tcccctagcg atagagcact 600

cgatcttccc agaaaaagag gcagaagcag tagcagaaca ggccacacaa tcgcaagtga 660

ttaacgtcca cacaggtata gggtttctgg accatatgat acatgctctg gccaagcatt 720

ccggctggtc gctaatcgtt gagtgcattg gtgacttaca catagacgac catcacacca 780

ctgaagactg cgggattgct ctcggtcaag cttttaaaga ggccctactg gcgcgtggag 840

taaaaaggtt tggatcagga tttgcgcctt tggatgaggc actttccaga gcggtggtag 900

atctttcgaa caggccgtac gcagttgtcg aacttggttt gcaaagggag aaagtaggag 960

atctctcttg cgagatgatc ccgcattttc ttgaaagctt tgcagaggct agcagaatta 1020

ccctccacgt tgattgtctg cgaggcaaga atgatcatca ccgtagtgag agtgcgttca 1080

aggctcttgc ggttgccata agagaagcca cctcgcccaa tggtaccaac gatgttccct 1140

ccaccaaagg tgttcttatg tagtgacacc gattatttaa agctgcagca tacgatatat 1200

atacatgtgt atatatgtat acctatgaat gtcagtaagt atgtatacga acagtatgat 1260

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actgaagatg acaaggtaat gcatcattct atacgtgtca ttctgaacga ggcgcgcttt   1320
ccttttttct ttttgctttt tcttttttt tctcttgaac tcgacggatc tatgcggtgt   1380
gaaataccgc acagatgcgt aaggagaaaa taccgcatca ggaaattgta aacgttaata   1440
ttttgttaaa attcgcgtta aatttttgtt aaatcagctc atttttaac caataggccg   1500
aaatcggcaa aatcccttat aaatcaaaag aatagaccga gatagggttg agtgttgttc   1560
cagtttggaa caagagtcca ctattaaaga acgtggactc caacgtcaaa gggcgaaaaa   1620
ccgtctatca gggcgatggc ccactacgtg aaccatcacc ctaatcaagt tttttggggt   1680
cgaggtgccg taaagcacta aatcggaacc ctaaagggag cccccgattt agagcttgac   1740
ggggaaagcc ggcgaacgtg gcgagaaagg aagggaagaa agcgaaagga gcgggcgcta   1800
gggcgctggc aagtgtagcg gtcacgctgc gcgtaaccac cacacccgcc gcgcttaatg   1860
cgccgctaca gggcgcgtcg cgccattcgc cattcaggct gcgcaactgt tgggaagggc   1920
gatcggtgcg ggcctcttcg ctattacgcc agctggcgaa aggggggatgt gctgcaaggc   1980
gattaagttg ggtaacgcca gggttttccc agtcacgacg ttgtaaaacg acggccagtg   2040
agcgcgcgta atacgactca ctatagggcg aattgggtac cgggcccccc ctcgaggtcg   2100
acggtatcga taagcttgat tagaagccgc cgagcgggcg acagccctcc gacggaagac   2160
tctcctccgt gcgtcctcgt cttcaccggt cgcgttcctg aaacgcagat gtgcctcgcg   2220
ccgcactgct ccgaacaata aagattctac aatactagct tttatggtta tgaagaggaa   2280
aaattggcag taacctggcc ccacaaacct tcaaattaac gaatcaaatt aacaaccata   2340
ggatgataat gcgattagtt ttttagcctt atttctgggg taattaatca gcgaagcgat   2400
gatttttgat ctattaacag atatataaat ggaaaagctg cataaccact ttaactaata   2460
ctttcaacat tttcagtttg tattacttct tattcaaatg tcataaaagt atcaacaaaa   2520
aattgttaat atacctctat actttaacgt caaggagaaa aatgtccaat ttactgcccg   2580
tacaccaaaa tttgcctgca ttaccggtcg atgcaacgag tgatgaggtt cgcaagaacc   2640
tgatggacat gttcagggat cgccaggcgt tttctgagca tacctggaaa atgcttctgt   2700
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aacctgaaga tgttcgcgat tatcttctat atcttcaggc gcgcggtctg gcagtaaaaa   2820
ctatccagca acatttgggc cagctaaaca tgcttcatcg tcggtccggg ctgccacgac   2880
caagtgacag caatgctgtt tcactggtta tgcggcggat ccgaaaagaa aacgttgatg   2940
ccggtgaacg tgcaaaacag gctctagcgt tcgaacgcac tgatttcgac caggttcgtt   3000
cactcatgga aaatagcgat cgctgccagg atatacgtaa tctggcattt ctggggattg   3060
cttataacac cctgttacgt atagccgaaa ttgccaggat cagggttaaa gatatctcac   3120
gtactgacgg tgggagaatg ttaatccata ttggcagaac gaaaacgctg gttagcaccg   3180
caggtgtaga gaaggcactt agcctggggg taactaaact ggtcgagcga tggatttccg   3240
tctctggtgt agctgatgat ccgaataact acctgttttg ccgggtcaga aaaaatggtg   3300
ttgccgcgcc atctgccacc agccagctat caactcgcgc cctggaaggg atttttgaag   3360
caactcatcg attgatttac ggcgctaagg atgactctgg tcagagatac ctggcctggt   3420
ctggacacag tgcccgtgtc ggagccgcgc gagatatggc ccgcgctgga gtttcaatac   3480
cggagatcat gcaagctggt ggctggacca atgtaaatat tgtcatgaac tatatccgta   3540
acctggatag tgaaacaggg gcaatggtgc gcctgctgga agatggcgat taggagtaag   3600
```

```
cgaatttctt atgatttatg atttttatta ttaaataagt tataaaaaaa ataagtgtat   3660
acaaatttta aagtgactct taggttttaa aacgaaaatt cttattcttg agtaactctt   3720
tcctgtaggt caggttgctt tctcaggtat agcatgaggt cgctcttatt gaccacacct   3780
ctaccggcat gccgagcaaa tgcctgcaaa tcgctcccca tttcacccaa ttgtagatat   3840
gctaactcca gcaatgagtt gatgaatctc ggtgtgtatt ttatgtcctc agaggacaac   3900
acctgtggtg ttctagagcg gccgccaccg cggtggagct ccagcttttg ttccctttag   3960
tgagggttaa ttgcgcgctt ggcgtaatca tggtcatagc tgtttcctgt gtgaaattgt   4020
tatccgctca caattccaca caacatagga gccggaagca taaagtgtaa agcctggggt   4080
gcctaatgag tgaggtaact cacattaatt gcgttgcgct cactgcccgc tttccagtcg   4140
ggaaacctgt cgtgccagct gcattaatga atcggccaac gcgcggggag aggcggtttg   4200
cgtattgggc gctcttccgc ttcctcgctc actgactcgc tgcgctcggt cgttcggctg   4260
cggcgagcgg tatcagctca ctcaaaggcg gtaatacggt tatccacaga atcaggggat   4320
aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc   4380
gcgttgctgg cgtttttcca taggctccgc ccccctgacg agcatcacaa aaatcgacgc   4440
tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga   4500
agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt   4560
ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg   4620
taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc   4680
gccttatccg gtaactatcg tcttgagtcc aacccggtaa gacacgactt atcgccactg   4740
gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc   4800
ttgaagtggt ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg   4860
ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc   4920
gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct   4980
caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt   5040
taagggattt tggtcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa   5100
aaatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa   5160
tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcatc catagttgcc   5220
tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg ccccagtgct   5280
gcaatgatac cgcgagaccc acgctcaccg gctccagatt tatcagcaat aaaccagcca   5340
gccggaaggg ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt   5400
aattgttgcc gggaagctag agtaagtagt tcgccagtta atagtttgcg caacgttgtt   5460
gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc   5520
ggttcccaac gatcaaggcg agttacatga tcccccatgt tgtgcaaaaa agcggttagc   5580
tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc actcatggtt   5640
atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact   5700
ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttgc   5760
ccggcgtcaa tacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt   5820
ggaaaacgtt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg   5880
atgtaaccca ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct   5940
gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa   6000
```

```
tgttgaatac tcatactctt ccttttttcaa tattattgaa gcatttatca gggttattgt   6060

ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc   6120

acatttcccc gaaaagtgcc acctgaacga agcatctgtg cttcattttg tagaacaaaa   6180

atgcaacgcg agagcgctaa tttttcaaac aaagaatctg agctgcattt ttacagaaca   6240

gaaatgcaac gcgaaagcgc tattttacca acgaagaatc tgtgcttcat ttttgtaaaa   6300

caaaaatgca acgcgagagc gctaattttt caaacaaaga atctgagctg catttttaca   6360

gaacagaaat gcaacgcgag agcgctattt taccaacaaa gaatctatac ttcttttttg   6420

ttctacaaaa atgcatcccg agagcgctat ttttctaaca aagcatctta gattactttt   6480

tttctccttt gtgcgctcta taatgcagtc tcttgataac tttttgcact gtaggtccgt   6540

taaggttaga agaaggctac tttggtgtct attttctctt ccataaaaaa agcctgactc   6600

cacttcccgc gtttactgat tactagcgaa gctgcgggtg catttttca agataaaggc   6660

atccccgatt atattctata ccgatgtgga ttgcgcatac tttgtgaaca gaaagtgata   6720

gcgttgatga ttcttcattg gtcagaaaat tatgaacggt ttcttctatt ttgtctctat   6780

atactacgta taggaaatgt ttacattttc gtattgtttt cgattcactc tatgaatagt   6840

tcttactaca attttttgt ctaaagagta atactagaga taaacataaa aaatgtagag   6900

gtcgagttta gatgcaagtt caaggagcga aaggtggatg ggtaggttat atagggatat   6960

agcacagaga tatatagcaa agagatactt ttgagcaatg tttgtggaag cggtattcgc   7020

aatattttag tagctcgtta cagtccggtg cgtttttggt tttttgaaag tgcgtcttca   7080

gagcgctttt ggttttcaaa agcgctctga agttcctata ctttctagag aataggaact   7140

tcggaatagg aacttcaaag cgtttccgaa aacgagcgct tccgaaaatg caacgcgagc   7200

tgcgcacata cagctcactg ttcacgtcgc acctatatct gcgtgttgcc tgtatatata   7260

tatacatgag aagaacggca tagtgcgtgt ttatgcttaa atgcgtactt atatgcgtct   7320

atttatgtag gatgaaaggt agtctagtac ctcctgtgat attatcccat tccatgcggg   7380

gtatcgtatg cttccttcag cactaccctt tagctgttct atatgctgcc actcctcaat   7440

tggattagtc tcatccttca atgctatcat ttcctttgat attggatcat ctaagaaacc   7500

attattatca tgacattaac ctataaaaat aggcgtatca cgaggccctt tcgtc        7555
```

<210> SEQ ID NO 122
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T001

<400> SEQUENCE: 122

```
tcaaggtacc atggcaagtt cgggcacaac                                      30
```

<210> SEQ ID NO 123
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T002

<400> SEQUENCE: 123

```
taacgcggcc gcttattccc ccaccatttc ag                                   32
```

<210> SEQ ID NO 124

<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T003

<400> SEQUENCE: 124

atcattgcat gcgcctactt ggcttcacat acgttg 36

<210> SEQ ID NO 125
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T004

<400> SEQUENCE: 125

catggtacct tgaatatgta ttacttgg 28

<210> SEQ ID NO 126
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T005

<400> SEQUENCE: 126

ataagcggcc gcgttaattc aaattaattg atatag 36

<210> SEQ ID NO 127
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T006

<400> SEQUENCE: 127

ttagattgga tcccgcgaac tccaaaatga gctatc 36

<210> SEQ ID NO 128
<211> LENGTH: 94
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T007

<400> SEQUENCE: 128

atgagagctt tggcatattt caagaagggt gatattcact tcactaatga tatccctagg 60

ccaggcctac ttggcttcac atacgttgca tacg 94

<210> SEQ ID NO 129
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer T008

<400> SEQUENCE: 129

ttacttcatt tcaccgtgat tgttaggcgt caatagaatc ttaacgttgg attccttgtg 60

caccttggct aactcgttgt atcatcactg g 91

<210> SEQ ID NO 130
<211> LENGTH: 2064
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 130

```
atgatcagac aatctacgct aaaaaacttc gctattaagc gttgctttca acatatagca      60
taccgcaaca cacctgccat gagatcagta gctctcgcgc agcgctttta tagttcgtct     120
tcccgttatt acagtgcgtc tccattacca gcctctaaaa ggccagagcc tgctccaagt     180
ttcaatgttg atccattaga acagcccgct gaaccttcaa aattggctaa gaaactacgc     240
gctgagcctg acatggatac ctctttcgtc ggtttaactg gtggtcaaat atttaacgaa     300
atgatgtcca gacaaaacgt tgatactgta tttggttatc caggtggtgc tatcctacct     360
gtttacgatg ccattcataa cagtgataaa ttcaacttcg ttcttccaaa acacgaacaa     420
ggtgccggtc acatggcaga aggctacgcc agagcttctg gtaaaccagg tgttgtcttg     480
gttacttctg ggccaggtgc caccaatgtc gttactccaa tggcagatgc ctttgcagac     540
gggattccaa tggttgtctt tacagggcaa gtcccaacta gtgctatcgg tactgatgct     600
ttccaagagg ctgacgtcgt tggtatttct agatcttgta cgaaatggaa tgtcatggtc     660
aagtccgtgg aagaattgcc attgcgtatt aacgaggctt ttgaaattgc cacgagcggt     720
agaccgggac cagtcttggt cgatttacca aaggatgtta cagcagctat cttaagaaat     780
ccaattccaa caaaaacaac tcttccatca aacgcactaa accaattaac cagtcgcgca     840
caagatgaat ttgtcatgca aagtatcaat aaagcagcag atttgatcaa cttggcaaag     900
aaacctgtct tatacgtcgg tgctggtatt ttaaaccatg cagatggtcc aagattacta     960
aaagaattaa gtgaccgtgc tcaaatacct gtcaccacta ctttacaagg tttaggttca    1020
ttcgaccaag aagatccaaa atcattggat atgcttggta tgcacggttg tgctactgcc    1080
aacctggcag tgcaaaatgc cgacttgata attgcagttg gtgctagatt cgacgaccgt    1140
gtcactggta atatttctaa attcgctcca gaagctcgtc gtgcagctgc cgagggtaga    1200
ggtggtatta ttcatttcga ggttagtcca aaaaacataa acaaggttgt tcaaactcaa    1260
atagcagtgg aaggtgatgc tacgaccaat ctgggcaaaa tgatgtcaaa gattttccca    1320
gttaaggaga ggtctgaatg gtttgctcaa ataaataaat ggaagaagga atacccatac    1380
gcttatatgg aggagactcc aggatctaaa attaaaccac agacggttat aaagaaacta    1440
tccaaggttg ccaacgacac aggaagacat gtcattgtta caacgggtgt ggggcaacat    1500
caaatgtggg ctgctcaaca ctggacatgg agaaatccac atactttcat cacatcaggt    1560
ggtttaggta cgatgggtta cggtctccct gccgccatcg gtgctcaagt tgcaaagcca    1620
gaatctttgg ttattgacat tgatggtgac gcatccttta acatgactct aacggaattg    1680
agttctgccg ttcaagctgg tactccagtg aagattttga ttttgaacaa tgaagagcaa    1740
ggtatggtta ctcaatggca atccctgttc tacgaacatc gttattccca cacacatcaa    1800
ttgaaccctg atttcataaa actagcggag gctatgggtt taaaaggttt aagagtcaag    1860
aagcaagagg aattggacgc taagttgaaa gaattcgttt ctaccaaggg cccagttttg    1920
cttgaagtgg aagttgataa aaaagttcct gttttgccaa tggtggcagg tggtagcggt    1980
ctagacgagt tcataaattt tgacccagaa gttgaaagac aacagactga attacgtcat    2040
aagcgtacag gcggtaagca ctga                                           2064
```

<210> SEQ ID NO 131
<211> LENGTH: 687
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 131

```
Met Ile Arg Gln Ser Thr Leu Lys Asn Phe Ala Ile Lys Arg Cys Phe
1               5                   10                  15

Gln His Ile Ala Tyr Arg Asn Thr Pro Ala Met Arg Ser Val Ala Leu
            20                  25                  30

Ala Gln Arg Phe Tyr Ser Ser Ser Ser Arg Tyr Tyr Ser Ala Ser Pro
        35                  40                  45

Leu Pro Ala Ser Lys Arg Pro Glu Pro Ala Pro Ser Phe Asn Val Asp
    50                  55                  60

Pro Leu Glu Gln Pro Ala Glu Pro Ser Lys Leu Ala Lys Lys Leu Arg
65                  70                  75                  80

Ala Glu Pro Asp Met Asp Thr Ser Phe Val Gly Leu Thr Gly Gly Gln
                85                  90                  95

Ile Phe Asn Glu Met Met Ser Arg Gln Asn Val Asp Thr Val Phe Gly
            100                 105                 110

Tyr Pro Gly Gly Ala Ile Leu Pro Val Tyr Asp Ala Ile His Asn Ser
        115                 120                 125

Asp Lys Phe Asn Phe Val Leu Pro Lys His Glu Gln Gly Ala Gly His
    130                 135                 140

Met Ala Glu Gly Tyr Ala Arg Ala Ser Gly Lys Pro Gly Val Val Leu
145                 150                 155                 160

Val Thr Ser Gly Pro Gly Ala Thr Asn Val Val Thr Pro Met Ala Asp
                165                 170                 175

Ala Phe Ala Asp Gly Ile Pro Met Val Val Phe Thr Gly Gln Val Pro
            180                 185                 190

Thr Ser Ala Ile Gly Thr Asp Ala Phe Gln Glu Ala Asp Val Val Gly
        195                 200                 205

Ile Ser Arg Ser Cys Thr Lys Trp Asn Val Met Val Lys Ser Val Glu
    210                 215                 220

Glu Leu Pro Leu Arg Ile Asn Glu Ala Phe Glu Ile Ala Thr Ser Gly
225                 230                 235                 240

Arg Pro Gly Pro Val Leu Val Asp Leu Pro Lys Asp Val Thr Ala Ala
                245                 250                 255

Ile Leu Arg Asn Pro Ile Pro Thr Lys Thr Thr Leu Pro Ser Asn Ala
            260                 265                 270

Leu Asn Gln Leu Thr Ser Arg Ala Gln Asp Glu Phe Val Met Gln Ser
        275                 280                 285

Ile Asn Lys Ala Ala Asp Leu Ile Asn Leu Ala Lys Lys Pro Val Leu
    290                 295                 300

Tyr Val Gly Ala Gly Ile Leu Asn His Ala Asp Gly Pro Arg Leu Leu
305                 310                 315                 320

Lys Glu Leu Ser Asp Arg Ala Gln Ile Pro Val Thr Thr Thr Leu Gln
                325                 330                 335

Gly Leu Gly Ser Phe Asp Gln Glu Asp Pro Lys Ser Leu Asp Met Leu
            340                 345                 350

Gly Met His Gly Cys Ala Thr Ala Asn Leu Ala Val Gln Asn Ala Asp
        355                 360                 365

Leu Ile Ile Ala Val Gly Ala Arg Phe Asp Asp Arg Val Thr Gly Asn
    370                 375                 380

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

Gly Gly Ile Ile His Phe Glu Val Ser Pro Lys Asn Ile Asn Lys Val
                405                 410                 415
```

```
Val Gln Thr Gln Ile Ala Val Glu Gly Asp Ala Thr Thr Asn Leu Gly
            420                 425                 430

Lys Met Met Ser Lys Ile Phe Pro Val Lys Glu Arg Ser Glu Trp Phe
        435                 440                 445

Ala Gln Ile Asn Lys Trp Lys Lys Glu Tyr Pro Tyr Ala Tyr Met Glu
    450                 455                 460

Glu Thr Pro Gly Ser Lys Ile Lys Pro Gln Thr Val Ile Lys Lys Leu
465                 470                 475                 480

Ser Lys Val Ala Asn Asp Thr Gly Arg His Val Ile Val Thr Thr Gly
                485                 490                 495

Val Gly Gln His Gln Met Trp Ala Ala Gln His Trp Thr Trp Arg Asn
            500                 505                 510

Pro His Thr Phe Ile Thr Ser Gly Gly Leu Gly Thr Met Gly Tyr Gly
        515                 520                 525

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

Ile Asp Ile Asp Gly Asp Ala Ser Phe Asn Met Thr Leu Thr Glu Leu
545                 550                 555                 560

Ser Ser Ala Val Gln Ala Gly Thr Pro Val Lys Ile Leu Ile Leu Asn
                565                 570                 575

Asn Glu Glu Gln Gly Met Val Thr Gln Trp Gln Ser Leu Phe Tyr Glu
            580                 585                 590

His Arg Tyr Ser His Thr His Gln Leu Asn Pro Asp Phe Ile Lys Leu
        595                 600                 605

Ala Glu Ala Met Gly Leu Lys Gly Leu Arg Val Lys Lys Gln Glu Glu
    610                 615                 620

Leu Asp Ala Lys Leu Lys Glu Phe Val Ser Thr Lys Gly Pro Val Leu
625                 630                 635                 640

Leu Glu Val Glu Val Asp Lys Lys Val Pro Val Leu Pro Met Val Ala
                645                 650                 655

Gly Gly Ser Gly Leu Asp Glu Phe Ile Asn Phe Asp Pro Glu Val Glu
            660                 665                 670

Arg Gln Gln Thr Glu Leu Arg His Lys Arg Thr Gly Gly Lys His
        675                 680                 685
```

<210> SEQ ID NO 132
<211> LENGTH: 1689
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 132

```
atggcaagtt cgggcacaac atcgacgcgt aagcgcttta ccggcgcaga atttatcgtt      60

catttcctgg aacagcaggg cattaagatt gtgacaggca ttccgggcgg ttctatcctg     120

cctgtttacg atgccttaag ccaaagcacg caaatccgcc atattctggc ccgtcatgaa     180

cagggcgcgg gctttatcgc tcagggaatg gcgcgcaccg acggtaaacc ggcggtctgt     240

atggcctgta gcggaccggg tgcgactaac ctggtgaccg ccattgccga tgcgcggctg     300

gactccatcc cgctgatttg catcactggt caggttcccg cctcgatgat cggcaccgac     360

gccttccagg aagtggacac ctacggcatc tctatcccca tcaccaaaca caactatctg     420

gtcagacata tcgaagaact cccgcaggtc atgagcgatg ccttccgcat tgcgcaatca     480

ggccgcccag gcccggtgtg gatagacatt cctaaggatg tgcaaacggc agtttttgag     540

attgaaacac agcccgctat ggcagaaaaa gccgccgccc ccgcctttag cgaagaaagc     600
```

```
attcgtgacg cagcggcgat gattaacgct gccaaacgcc cggtgcttta tctgggcggc    660

ggtgtgatca atgcgcccgc acgggtgcgt gaactggcgg agaaagcgca actgcctacc    720

accatgactt taatggcgct gggcatgttg ccaaaagcgc atccgttgtc gctgggtatg    780

ctggggatgc acggcgtgcg cagcaccaac tatattttgc aggaggcgga tttgttgata    840

gtgctcggtg cgcgttttga tgaccgggcg attggcaaaa ccgagcagtt ctgtccgaat    900

gccaaaatca ttcatgtcga tatcgaccgt gcagagctgg gtaaaatcaa gcagccgcac    960

gtggcgattc aggcggatgt tgatgacgtg ctggcgcagt tgatcccgct ggtggaagcg   1020

caaccgcgtg cagagtggca ccagttggta gcggatttgc agcgtgagtt tccgtgtcca   1080

atcccgaaag cgtgcgatcc gttaagccat tacggcctga tcaacgccgt tgccgcctgt   1140

gtcgatgaca atgcaattat caccaccgac gttggtcagc atcagatgtg gaccgcgcaa   1200

gcttatccgc tcaatcgccc acgccagtgg ctgacctccg gtgggctggg cacgatgggt   1260

tttggcctgc ctgcggcgat tggcgctgcg ctggcgaacc cggatcgcaa agtgttgtgt   1320

ttctccggcg acggcagcct gatgatgaat attcaggaga tggcgaccgc cagtgaaaat   1380

cagctggatg tcaaaatcat tctgatgaac aacgaagcgc tggggctggt gcatcagcaa   1440

cagagtctgt tctacgagca aggcgttttt gccgccacct atccgggcaa aatcaacttt   1500

atgcagattg ccgccggatt cggcctcgaa acctgtgatt tgaataacga agccgatccg   1560

caggcttcat tgcaggaaat catcaatcgc cctggcccgg cgctgatcca tgtgcgcatt   1620

gatgccgaag aaaaagttta cccgatggtg ccgccaggtg cggcgaatac tgaaatggtg   1680

ggggaataa                                                           1689
```

```
<210> SEQ ID NO 133
<211> LENGTH: 562
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 133

Met Ala Ser Ser Gly Thr Thr Ser Thr Arg Lys Arg Phe Thr Gly Ala
1               5                   10                  15

Glu Phe Ile Val His Phe Leu Glu Gln Gln Gly Ile Lys Ile Val Thr
            20                  25                  30

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

Ser Thr Gln Ile Arg His Ile Leu Ala Arg His Glu Gln Gly Ala Gly
    50                  55                  60

Phe Ile Ala Gln Gly Met Ala Arg Thr Asp Gly Lys Pro Ala Val Cys
65                  70                  75                  80

Met Ala Cys Ser Gly Pro Gly Ala Thr Asn Leu Val Thr Ala Ile Ala
                85                  90                  95

Asp Ala Arg Leu Asp Ser Ile Pro Leu Ile Cys Ile Thr Gly Gln Val
            100                 105                 110

Pro Ala Ser Met Ile Gly Thr Asp Ala Phe Gln Glu Val Asp Thr Tyr
        115                 120                 125

Gly Ile Ser Ile Pro Ile Thr Lys His Asn Tyr Leu Val Arg His Ile
    130                 135                 140

Glu Glu Leu Pro Gln Val Met Ser Asp Ala Phe Arg Ile Ala Gln Ser
145                 150                 155                 160

Gly Arg Pro Gly Pro Val Trp Ile Asp Ile Pro Lys Asp Val Gln Thr
                165                 170                 175
```

```
Ala Val Phe Glu Ile Glu Thr Gln Pro Ala Met Ala Glu Lys Ala Ala
            180                 185                 190

Ala Pro Ala Phe Ser Glu Glu Ser Ile Arg Asp Ala Ala Ala Met Ile
        195                 200                 205

Asn Ala Ala Lys Arg Pro Val Leu Tyr Leu Gly Gly Gly Val Ile Asn
    210                 215                 220

Ala Pro Ala Arg Val Arg Glu Leu Ala Glu Lys Ala Gln Leu Pro Thr
225                 230                 235                 240

Thr Met Thr Leu Met Ala Leu Gly Met Leu Pro Lys Ala His Pro Leu
                245                 250                 255

Ser Leu Gly Met Leu Gly Met His Gly Val Arg Ser Thr Asn Tyr Ile
            260                 265                 270

Leu Gln Glu Ala Asp Leu Leu Ile Val Leu Gly Ala Arg Phe Asp Asp
        275                 280                 285

Arg Ala Ile Gly Lys Thr Glu Gln Phe Cys Pro Asn Ala Lys Ile Ile
    290                 295                 300

His Val Asp Ile Asp Arg Ala Glu Leu Gly Lys Ile Lys Gln Pro His
305                 310                 315                 320

Val Ala Ile Gln Ala Asp Val Asp Asp Val Leu Ala Gln Leu Ile Pro
                325                 330                 335

Leu Val Glu Ala Gln Pro Arg Ala Glu Trp His Gln Leu Val Ala Asp
            340                 345                 350

Leu Gln Arg Glu Phe Pro Cys Pro Ile Pro Lys Ala Cys Asp Pro Leu
        355                 360                 365

Ser His Tyr Gly Leu Ile Asn Ala Val Ala Ala Cys Val Asp Asp Asn
    370                 375                 380

Ala Ile Ile Thr Thr Asp Val Gly Gln His Gln Met Trp Thr Ala Gln
385                 390                 395                 400

Ala Tyr Pro Leu Asn Arg Pro Arg Gln Trp Leu Thr Ser Gly Gly Leu
                405                 410                 415

Gly Thr Met Gly Phe Gly Leu Pro Ala Ala Ile Gly Ala Ala Leu Ala
            420                 425                 430

Asn Pro Asp Arg Lys Val Leu Cys Phe Ser Gly Asp Gly Ser Leu Met
        435                 440                 445

Met Asn Ile Gln Glu Met Ala Thr Ala Ser Glu Asn Gln Leu Asp Val
    450                 455                 460

Lys Ile Ile Leu Met Asn Asn Glu Ala Leu Gly Leu Val His Gln Gln
465                 470                 475                 480

Gln Ser Leu Phe Tyr Glu Gln Gly Val Phe Ala Ala Thr Tyr Pro Gly
                485                 490                 495

Lys Ile Asn Phe Met Gln Ile Ala Ala Gly Phe Gly Leu Glu Thr Cys
            500                 505                 510

Asp Leu Asn Asn Glu Ala Asp Pro Gln Ala Ser Leu Gln Glu Ile Ile
        515                 520                 525

Asn Arg Pro Gly Pro Ala Leu Ile His Val Arg Ile Asp Ala Glu Glu
    530                 535                 540

Lys Val Tyr Pro Met Val Pro Pro Gly Ala Ala Asn Thr Glu Met Val
545                 550                 555                 560

Gly Glu
```

<210> SEQ ID NO 134
<211> LENGTH: 338
<212> TYPE: PRT

<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 134

Met Lys Val Phe Tyr Asp Lys Asp Cys Asp Leu Ser Ile Ile Gln Gly
1 5 10 15

Lys Lys Val Ala Ile Ile Gly Tyr Gly Ser Gln Gly His Ala Gln Ala
20 25 30

Cys Asn Leu Lys Asp Ser Gly Val Asp Val Thr Val Gly Leu Arg Lys
35 40 45

Gly Ser Ala Thr Val Ala Lys Ala Glu Ala His Gly Leu Lys Val Thr
50 55 60

Asp Val Ala Ala Ala Val Ala Gly Ala Asp Leu Val Met Ile Leu Thr
65 70 75 80

Pro Asp Glu Phe Gln Ser Gln Leu Tyr Lys Asn Glu Ile Glu Pro Asn
85 90 95

Ile Lys Lys Gly Ala Thr Leu Ala Phe Ser His Gly Phe Ala Ile His
100 105 110

Tyr Asn Gln Val Val Pro Arg Ala Asp Leu Asp Val Ile Met Ile Ala
115 120 125

Pro Lys Ala Pro Gly His Thr Val Arg Ser Glu Phe Val Lys Gly Gly
130 135 140

Gly Ile Pro Asp Leu Ile Ala Ile Tyr Gln Asp Ala Ser Gly Asn Ala
145 150 155 160

Lys Asn Val Ala Leu Ser Tyr Ala Ala Gly Val Gly Gly Gly Arg Thr
165 170 175

Gly Ile Ile Glu Thr Thr Phe Lys Asp Glu Thr Glu Thr Asp Leu Phe
180 185 190

Gly Glu Gln Ala Val Leu Cys Gly Gly Thr Val Glu Leu Val Lys Ala
195 200 205

Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr Ala Pro Glu Met Ala Tyr
210 215 220

Phe Glu Cys Leu His Glu Leu Lys Leu Ile Val Asp Leu Met Tyr Glu
225 230 235 240

Gly Gly Ile Ala Asn Met Asn Tyr Ser Ile Ser Asn Asn Ala Glu Tyr
245 250 255

Gly Glu Tyr Val Thr Gly Pro Glu Val Ile Asn Ala Glu Ser Arg Gln
260 265 270

Ala Met Arg Asn Ala Leu Lys Arg Ile Gln Asp Gly Glu Tyr Ala Lys
275 280 285

Met Phe Ile Ser Glu Gly Ala Thr Gly Tyr Pro Ser Met Thr Ala Lys
290 295 300

Arg Arg Asn Asn Ala Ala His Gly Ile Glu Ile Ile Gly Glu Gln Leu
305 310 315 320

Arg Ser Met Met Pro Trp Ile Gly Ala Asn Lys Ile Val Asp Lys Ala
325 330 335

Lys Asn

<210> SEQ ID NO 135
<211> LENGTH: 571
<212> TYPE: PRT
<213> ORGANISM: Streptococcus mutans

<400> SEQUENCE: 135

Met Thr Asp Lys Lys Thr Leu Lys Asp Leu Arg Asn Arg Ser Ser Val
1 5 10 15

```
Tyr Asp Ser Met Val Lys Ser Pro Asn Arg Ala Met Leu Arg Ala Thr
            20                  25                  30

Gly Met Gln Asp Glu Asp Phe Glu Lys Pro Ile Val Gly Val Ile Ser
        35                  40                  45

Thr Trp Ala Glu Asn Thr Pro Cys Asn Ile His Leu His Asp Phe Gly
    50                  55                  60

Lys Leu Ala Lys Val Gly Val Lys Glu Ala Gly Ala Trp Pro Val Gln
65                  70                  75                  80

Phe Gly Thr Ile Thr Val Ser Asp Gly Ile Ala Met Gly Thr Gln Gly
                85                  90                  95

Met Arg Phe Ser Leu Thr Ser Arg Asp Ile Ile Ala Asp Ser Ile Glu
            100                 105                 110

Ala Ala Met Gly Gly His Asn Ala Asp Ala Phe Val Ala Ile Gly Gly
        115                 120                 125

Cys Asp Lys Asn Met Pro Gly Ser Val Ile Ala Met Ala Asn Met Asp
    130                 135                 140

Ile Pro Ala Ile Phe Ala Tyr Gly Gly Thr Ile Ala Pro Gly Asn Leu
145                 150                 155                 160

Asp Gly Lys Asp Ile Asp Leu Val Ser Val Phe Glu Gly Val Gly His
                165                 170                 175

Trp Asn His Gly Asp Met Thr Lys Glu Glu Val Lys Ala Leu Glu Cys
            180                 185                 190

Asn Ala Cys Pro Gly Pro Gly Gly Cys Gly Gly Met Tyr Thr Ala Asn
        195                 200                 205

Thr Met Ala Thr Ala Ile Glu Val Leu Gly Leu Ser Leu Pro Gly Ser
    210                 215                 220

Ser Ser His Pro Ala Glu Ser Ala Glu Lys Lys Ala Asp Ile Glu Glu
225                 230                 235                 240

Ala Gly Arg Ala Val Val Lys Met Leu Glu Met Gly Leu Lys Pro Ser
                245                 250                 255

Asp Ile Leu Thr Arg Glu Ala Phe Glu Asp Ala Ile Thr Val Thr Met
            260                 265                 270

Ala Leu Gly Gly Ser Thr Asn Ser Thr Leu His Leu Leu Ala Ile Ala
        275                 280                 285

His Ala Ala Asn Val Glu Leu Thr Leu Asp Asp Phe Asn Thr Phe Gln
    290                 295                 300

Glu Lys Val Pro His Leu Ala Asp Leu Lys Pro Ser Gly Gln Tyr Val
305                 310                 315                 320

Phe Gln Asp Leu Tyr Lys Val Gly Gly Val Pro Ala Val Met Lys Tyr
                325                 330                 335

Leu Leu Lys Asn Gly Phe Leu His Gly Asp Arg Ile Thr Cys Thr Gly
            340                 345                 350

Lys Thr Val Ala Glu Asn Leu Lys Ala Phe Asp Asp Leu Thr Pro Gly
        355                 360                 365

Gln Lys Val Ile Met Pro Leu Glu Asn Pro Lys Arg Glu Asp Gly Pro
    370                 375                 380

Leu Ile Ile Leu His Gly Asn Leu Ala Pro Asp Gly Ala Val Ala Lys
385                 390                 395                 400

Val Ser Gly Val Lys Val Arg Arg His Val Gly Pro Ala Lys Val Phe
                405                 410                 415

Asn Ser Glu Glu Glu Ala Ile Glu Ala Val Leu Asn Asp Asp Ile Val
            420                 425                 430
```

```
Asp Gly Asp Val Val Val Val Arg Phe Val Gly Pro Lys Gly Gly Pro
        435                 440                 445

Gly Met Pro Glu Met Leu Ser Leu Ser Ser Met Ile Val Gly Lys Gly
    450                 455                 460

Gln Gly Glu Lys Val Ala Leu Leu Thr Asp Gly Arg Phe Ser Gly Gly
465                 470                 475                 480

Thr Tyr Gly Leu Val Val Gly His Ile Ala Pro Glu Ala Gln Asp Gly
                485                 490                 495

Gly Pro Ile Ala Tyr Leu Gln Thr Gly Asp Ile Val Thr Ile Asp Gln
            500                 505                 510

Asp Thr Lys Glu Leu His Phe Asp Ile Ser Asp Glu Glu Leu Lys His
        515                 520                 525

Arg Gln Glu Thr Ile Glu Leu Pro Pro Leu Tyr Ser Arg Gly Ile Leu
    530                 535                 540

Gly Lys Tyr Ala His Ile Val Ser Ser Ala Ser Arg Gly Ala Val Thr
545                 550                 555                 560

Asp Phe Trp Lys Pro Glu Glu Thr Gly Lys Lys
                565                 570


<210> SEQ ID NO 136
<211> LENGTH: 548
<212> TYPE: PRT
<213> ORGANISM: Listeria grayi

<400> SEQUENCE: 136

Met Tyr Thr Val Gly Gln Tyr Leu Val Asp Arg Leu Glu Glu Ile Gly
1               5                   10                  15

Ile Asp Lys Val Phe Gly Val Pro Gly Asp Tyr Asn Leu Thr Phe Leu
            20                  25                  30

Asp Tyr Ile Gln Asn His Glu Gly Leu Ser Trp Gln Gly Asn Thr Asn
        35                  40                  45

Glu Leu Asn Ala Ala Tyr Ala Ala Asp Gly Tyr Ala Arg Glu Arg Gly
    50                  55                  60

Val Ser Ala Leu Val Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Ile
65                  70                  75                  80

Asn Gly Thr Ala Gly Ser Phe Ala Glu Gln Val Pro Val Ile His Ile
                85                  90                  95

Val Gly Ser Pro Thr Met Asn Val Gln Ser Asn Lys Lys Leu Val His
            100                 105                 110

His Ser Leu Gly Met Gly Asn Phe His Asn Phe Ser Glu Met Ala Lys
        115                 120                 125

Glu Val Thr Ala Ala Thr Thr Met Leu Thr Glu Glu Asn Ala Ala Ser
    130                 135                 140

Glu Ile Asp Arg Val Leu Glu Thr Ala Leu Leu Glu Lys Arg Pro Val
145                 150                 155                 160

Tyr Ile Asn Leu Pro Ile Asp Ile Ala His Lys Ala Ile Val Lys Pro
                165                 170                 175

Ala Lys Ala Leu Gln Thr Glu Lys Ser Ser Gly Glu Arg Glu Ala Gln
            180                 185                 190

Leu Ala Glu Ile Ile Leu Ser His Leu Glu Lys Ala Ala Gln Pro Ile
        195                 200                 205

Val Ile Ala Gly His Glu Ile Ala Arg Phe Gln Ile Arg Glu Arg Phe
    210                 215                 220

Glu Asn Trp Ile Asn Gln Thr Lys Leu Pro Val Thr Asn Leu Ala Tyr
225                 230                 235                 240
```

```
Gly Lys Gly Ser Phe Asn Glu Glu Asn Glu His Phe Ile Gly Thr Tyr
                245                 250                 255

Tyr Pro Ala Phe Ser Asp Lys Asn Val Leu Asp Tyr Val Asp Asn Ser
            260                 265                 270

Asp Phe Val Leu His Phe Gly Gly Lys Ile Ile Asp Asn Ser Thr Ser
        275                 280                 285

Ser Phe Ser Gln Gly Phe Lys Thr Glu Asn Thr Leu Thr Ala Ala Asn
    290                 295                 300

Asp Ile Ile Met Leu Pro Asp Gly Ser Thr Tyr Ser Gly Ile Ser Leu
305                 310                 315                 320

Asn Gly Leu Leu Ala Glu Leu Glu Lys Leu Asn Phe Thr Phe Ala Asp
                325                 330                 335

Thr Ala Ala Lys Gln Ala Glu Leu Ala Val Phe Glu Pro Gln Ala Glu
            340                 345                 350

Thr Pro Leu Lys Gln Asp Arg Phe His Gln Ala Val Met Asn Phe Leu
        355                 360                 365

Gln Ala Asp Asp Val Leu Val Thr Glu Gln Gly Thr Ser Ser Phe Gly
    370                 375                 380

Leu Met Leu Ala Pro Leu Lys Lys Gly Met Asn Leu Ile Ser Gln Thr
385                 390                 395                 400

Leu Trp Gly Ser Ile Gly Tyr Thr Leu Pro Ala Met Ile Gly Ser Gln
                405                 410                 415

Ile Ala Ala Pro Glu Arg Arg His Ile Leu Ser Ile Gly Asp Gly Ser
            420                 425                 430

Phe Gln Leu Thr Ala Gln Glu Met Ser Thr Ile Phe Arg Glu Lys Leu
        435                 440                 445

Thr Pro Val Ile Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg
    450                 455                 460

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

Leu Gln Leu Val Ala Glu Thr Phe Gly Gly Asp Ala Glu Thr Val Asp
                485                 490                 495

Thr His Asn Val Phe Thr Glu Thr Asp Phe Ala Asn Thr Leu Ala Ala
            500                 505                 510

Ile Asp Ala Thr Pro Gln Lys Ala His Val Val Glu Val His Met Glu
        515                 520                 525

Gln Met Asp Met Pro Glu Ser Leu Arg Gln Ile Gly Leu Ala Leu Ser
    530                 535                 540

Lys Gln Asn Ser
545
```

<210> SEQ ID NO 137
<211> LENGTH: 1641
<212> TYPE: DNA
<213> ORGANISM: Macrococcus caseolyticus

<400> SEQUENCE: 137

```
atgaaacaac gtatcgggca atacttgatc gatgccctac acgttaatgg tgtcgataag      60

atctttggag tcccaggtga tttcacttta gccttttttgg acgatatcat aagacatgac    120

aacgtggaat gggtgggaaa tactaatgag ttgaacgccg cttacgccgc tgatggttac     180

gctagagtta atggattagc cgctgtatct accacttttg ggttggcgga gttatctgct     240

gtgaatggta ttgctggaag ttacgcagag cgtgttcctg taatcaaaat ctcaggcggt     300
```

```
ccttcatcag ttgctcaaca agagggtaga tatgtccacc attcattggg tgaaggaatc    360
tttgattcat attcaaagat gtacgctcac ataaccgcaa caactacaat cttatccgtt    420
gacaacgcag tcgacgaaat tgatagagtt attcattgtg ctttgaagga aaagaggcca    480
gtgcatattc atttgcctat tgacgtagcc ttaactgaga ttgaaatccc tcatgcacca    540
aaagtttaca cacacgaatc ccagaacgtc gatgcttaca ttcaagctgt tgagaaaaag    600
ttaatgtctg caaaacaacc agtaatcata gcaggtcatg aaatcaattc attcaagttg    660
cacgaacaac tggaacagtt tgtcaatcag acaaacatcc ctgttgcaca actttccttg    720
ggtaagtctg ctttcaatga agagaatgaa cattaccttg gtatctacga tggcaaaatc    780
gcaaaggaaa atgtgagaga gtacgtcgac aatgctgatg tcatattgaa cataggtgcc    840
aaactgactg attctgctac agctggattt tcctacaagt tcgatacaaa caacataatc    900
tacattaacc ataatgactt caaagctgaa gatgtgattt ctgataatgt ttcactgatt    960
gatcttgtga atggcctgaa ttctattgac tatagaaatg aaacacacta cccatcttat   1020
caaagatctg atatgaaata cgaattgaat gacgcaccac ttacacaatc taactatttc   1080
aaaatgatga acgcttttct agaaaaagat gacatcctac tagctgaaca aggtacatcc   1140
tttttcggcg catatgactt atccctatac aagggaaatc agtttatcgg tcagccttta   1200
tgggggtcaa tagggtatac ttttccatct ttactaggaa gtcaactagc agacatgcat   1260
aggagaaaca ttttgcttat aggcgatggt agtttacaac ttactgttca agccctaagt   1320
acaatgatta gaaaggatat caaaccaatc attttcgtta tcaataacga cggttacacc   1380
gtcgaaagac ttatccacgg catggaagag ccatacaatg atatccaaat gtggaactac   1440
aagcaattgc cagaagtatt tggtggaaaa gatactgtaa aagttcatga tgctaaaacc   1500
tccaacgaac tgaaaactgt aatggattct gttaaagcag acaaagatca catgcatttc   1560
attgaagtgc atatggcagt agaggacgcc ccaaagaagt tgattgatat agctaaagcc   1620
tttagtgatg ctaacaagta a                                             1641
```

<210> SEQ ID NO 138
<211> LENGTH: 347
<212> TYPE: PRT
<213> ORGANISM: Beijerickia indica

<400> SEQUENCE: 138

```
Met Lys Ala Leu Val Tyr Arg Gly Pro Gly Gln Lys Leu Val Glu Glu
1               5                   10                  15

Arg Gln Lys Pro Glu Leu Lys Glu Pro Gly Asp Ala Ile Val Lys Val
            20                  25                  30

Thr Lys Thr Thr Ile Cys Gly Thr Asp Leu His Ile Leu Lys Gly Asp
        35                  40                  45

Val Ala Thr Cys Lys Pro Gly Arg Val Leu Gly His Glu Gly Val Gly
    50                  55                  60

Val Ile Glu Ser Val Gly Ser Gly Val Thr Ala Phe Gln Pro Gly Asp
65                  70                  75                  80

Arg Val Leu Ile Ser Cys Ile Ser Ser Cys Gly Lys Cys Ser Phe Cys
                85                  90                  95

Arg Arg Gly Met Phe Ser His Cys Thr Thr Gly Gly Trp Ile Leu Gly
            100                 105                 110

Asn Glu Ile Asp Gly Thr Gln Ala Glu Tyr Val Arg Val Pro His Ala
        115                 120                 125

Asp Thr Ser Leu Tyr Arg Ile Pro Ala Gly Ala Asp Glu Glu Ala Leu
```

```
    130                 135                 140

Val Met Leu Ser Asp Ile Leu Pro Thr Gly Phe Glu Cys Gly Val Leu
145                 150                 155                 160

Asn Gly Lys Val Ala Pro Gly Ser Ser Val Ala Ile Val Gly Ala Gly
                165                 170                 175

Pro Val Gly Leu Ala Ala Leu Leu Thr Ala Gln Phe Tyr Ser Pro Ala
            180                 185                 190

Glu Ile Ile Met Ile Asp Leu Asp Asp Asn Arg Leu Gly Leu Ala Lys
        195                 200                 205

Gln Phe Gly Ala Thr Arg Thr Val Asn Ser Thr Gly Gly Asn Ala Ala
    210                 215                 220

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

Glu Ala Val Gly Ile Pro Ala Thr Phe Glu Leu Cys Gln Asn Ile Val
                245                 250                 255

Ala Pro Gly Gly Thr Ile Ala Asn Val Gly Val His Gly Ser Lys Val
            260                 265                 270

Asp Leu His Leu Glu Ser Leu Trp Ser His Asn Val Thr Ile Thr Thr
        275                 280                 285

Arg Leu Val Asp Thr Ala Thr Thr Pro Met Leu Leu Lys Thr Val Gln
    290                 295                 300

Ser His Lys Leu Asp Pro Ser Arg Leu Ile Thr His Arg Phe Ser Leu
305                 310                 315                 320

Asp Gln Ile Leu Asp Ala Tyr Glu Thr Phe Gly Gln Ala Ala Ser Thr
                325                 330                 335

Gln Ala Leu Lys Val Ile Ile Ser Met Glu Ala
            340                 345


<210> SEQ ID NO 139
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Achromobacter xylosoxidans

<400> SEQUENCE: 139

Met Lys Ala Leu Val Tyr His Gly Asp His Lys Ile Ser Leu Glu Asp
1               5                   10                  15

Lys Pro Lys Pro Thr Leu Gln Lys Pro Thr Asp Val Val Val Arg Val
            20                  25                  30

Leu Lys Thr Thr Ile Cys Gly Thr Asp Leu Gly Ile Tyr Lys Gly Lys
        35                  40                  45

Asn Pro Glu Val Ala Asp Gly Arg Ile Leu Gly His Glu Gly Val Gly
    50                  55                  60

Val Ile Glu Glu Val Gly Glu Ser Val Thr Gln Phe Lys Lys Gly Asp
65                  70                  75                  80

Lys Val Leu Ile Ser Cys Val Thr Ser Cys Gly Ser Cys Asp Tyr Cys
                85                  90                  95

Lys Lys Gln Leu Tyr Ser His Cys Arg Asp Gly Gly Trp Ile Leu Gly
            100                 105                 110

Tyr Met Ile Asp Gly Val Gln Ala Glu Tyr Val Arg Ile Pro His Ala
        115                 120                 125

Asp Asn Ser Leu Tyr Lys Ile Pro Gln Thr Ile Asp Asp Glu Ile Ala
    130                 135                 140

Val Leu Leu Ser Asp Ile Leu Pro Thr Gly His Glu Ile Gly Val Gln
145                 150                 155                 160
```

```
Tyr Gly Asn Val Gln Pro Gly Asp Ala Val Ala Ile Val Gly Ala Gly
                165                 170                 175

Pro Val Gly Met Ser Val Leu Leu Thr Ala Gln Phe Tyr Ser Pro Ser
            180                 185                 190

Thr Ile Ile Val Ile Asp Met Asp Glu Asn Arg Leu Gln Leu Ala Lys
        195                 200                 205

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

Glu Ala Val His Arg Ile Ala Ala Glu Gly Val Asp Val Ala Ile Glu
225                 230                 235                 240

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

Pro Gly Ala His Ile Ala Asn Val Gly Val His Gly Val Lys Val Asp
            260                 265                 270

Phe Glu Ile Gln Lys Leu Trp Ile Lys Asn Leu Thr Ile Thr Thr Gly
        275                 280                 285

Leu Val Asn Thr Asn Thr Thr Pro Met Leu Met Lys Val Ala Ser Thr
    290                 295                 300

Asp Lys Leu Pro Leu Lys Lys Met Ile Thr His Arg Phe Glu Leu Ala
305                 310                 315                 320

Glu Ile Glu His Ala Tyr Gln Val Phe Leu Asn Gly Ala Lys Glu Lys
                325                 330                 335

Ala Met Lys Ile Ile Leu Ser Asn Ala Gly Ala Ala
            340                 345


<210> SEQ ID NO 140

<400> SEQUENCE: 140

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<210> SEQ ID NO 141
<211> LENGTH: 548
<212> TYPE: PRT
<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 141

Met Tyr Thr Val Gly Asp Tyr Leu Leu Asp Arg Leu His Glu Leu Gly
1               5                   10                  15

Ile Glu Glu Ile Phe Gly Val Pro Gly Asp Tyr Asn Leu Gln Phe Leu
            20                  25                  30

Asp Gln Ile Ile Ser His Lys Asp Met Lys Trp Val Gly Asn Ala Asn
        35                  40                  45

Glu Leu Asn Ala Ser Tyr Met Ala Asp Gly Tyr Ala Arg Thr Lys Lys
    50                  55                  60

Ala Ala Ala Phe Leu Thr Thr Phe Gly Val Gly Glu Leu Ser Ala Val
65                  70                  75                  80

Asn Gly Leu Ala Gly Ser Tyr Ala Glu Asn Leu Pro Val Val Glu Ile
                85                  90                  95

Val Gly Ser Pro Thr Ser Lys Val Gln Asn Glu Gly Lys Phe Val His
            100                 105                 110

His Thr Leu Ala Asp Gly Asp Phe Lys His Phe Met Lys Met His Glu
        115                 120                 125

Pro Val Thr Ala Ala Arg Thr Leu Leu Thr Ala Glu Asn Ala Thr Val
    130                 135                 140

Glu Ile Asp Arg Val Leu Ser Ala Leu Leu Lys Glu Arg Lys Pro Val
```

```
145                 150                 155                 160

Tyr Ile Asn Leu Pro Val Asp Val Ala Ala Ala Lys Ala Glu Lys Pro
                165                 170                 175

Ser Leu Pro Leu Lys Lys Glu Asn Ser Thr Ser Asn Thr Ser Asp Gln
            180                 185                 190

Glu Ile Leu Asn Lys Ile Gln Glu Ser Leu Lys Asn Ala Lys Lys Pro
        195                 200                 205

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

Val Thr Gln Phe Ile Ser Lys Thr Lys Leu Pro Ile Thr Thr Leu Asn
225                 230                 235                 240

Phe Gly Lys Ser Ser Val Asp Glu Ala Leu Pro Ser Phe Leu Gly Ile
                245                 250                 255

Tyr Asn Gly Thr Leu Ser Glu Pro Asn Leu Lys Glu Phe Val Glu Ser
            260                 265                 270

Ala Asp Phe Ile Leu Met Leu Gly Val Lys Leu Thr Asp Ser Ser Thr
        275                 280                 285

Gly Ala Phe Thr His His Leu Asn Glu Asn Lys Met Ile Ser Leu Asn
    290                 295                 300

Ile Asp Glu Gly Lys Ile Phe Asn Glu Arg Ile Gln Asn Phe Asp Phe
305                 310                 315                 320

Glu Ser Leu Ile Ser Ser Leu Leu Asp Leu Ser Glu Ile Glu Tyr Lys
                325                 330                 335

Gly Lys Tyr Ile Asp Lys Lys Gln Glu Asp Phe Val Pro Ser Asn Ala
            340                 345                 350

Leu Leu Ser Gln Asp Arg Leu Trp Gln Ala Val Glu Asn Leu Thr Gln
        355                 360                 365

Ser Asn Glu Thr Ile Val Ala Glu Gln Gly Thr Ser Phe Phe Gly Ala
    370                 375                 380

Ser Ser Ile Phe Leu Lys Ser Lys Ser His Phe Ile Gly Gln Pro Leu
385                 390                 395                 400

Trp Gly Ser Ile Gly Tyr Thr Phe Pro Ala Ala Leu Gly Ser Gln Ile
                405                 410                 415

Ala Asp Lys Glu Ser Arg His Leu Leu Phe Ile Gly Asp Gly Ser Leu
            420                 425                 430

Gln Leu Thr Val Gln Glu Leu Gly Leu Ala Ile Arg Glu Lys Ile Asn
        435                 440                 445

Pro Ile Cys Phe Ile Ile Asn Asn Asp Gly Tyr Thr Val Glu Arg Glu
    450                 455                 460

Ile His Gly Pro Asn Gln Ser Tyr Asn Asp Ile Pro Met Trp Asn Tyr
465                 470                 475                 480

Ser Lys Leu Pro Glu Ser Phe Gly Ala Thr Glu Asp Arg Val Val Ser
                485                 490                 495

Lys Ile Val Arg Thr Glu Asn Glu Phe Val Ser Val Met Lys Glu Ala
            500                 505                 510

Gln Ala Asp Pro Asn Arg Met Tyr Trp Ile Glu Leu Ile Leu Ala Lys
        515                 520                 525

Glu Gly Ala Pro Lys Val Leu Lys Lys Met Gly Lys Leu Phe Ala Glu
    530                 535                 540

Gln Asn Lys Ser
545

<210> SEQ ID NO 142
```

```
<211> LENGTH: 375
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

<400> SEQUENCE: 142

Met Ser Thr Ala Gly Lys Val Ile Lys Cys Lys Ala Ala Val Leu Trp
1               5                   10                  15

Glu Glu Lys Lys Pro Phe Ser Ile Glu Glu Val Glu Val Ala Pro Pro
            20                  25                  30

Lys Ala His Glu Val Arg Ile Lys Met Val Ala Thr Gly Ile Cys Arg
        35                  40                  45

Ser Asp Asp His Val Val Ser Gly Thr Leu Val Thr Pro Leu Pro Val
    50                  55                  60

Ile Ala Gly His Glu Ala Ala Gly Ile Val Glu Ser Ile Gly Glu Gly
65                  70                  75                  80

Val Thr Thr Val Arg Pro Gly Asp Lys Val Ile Pro Leu Phe Thr Pro
                85                  90                  95

Gln Cys Gly Lys Cys Arg Val Cys Lys His Pro Glu Gly Asn Phe Cys
            100                 105                 110

Leu Lys Asn Asp Leu Ser Met Pro Arg Gly Thr Met Gln Asp Gly Thr
        115                 120                 125

Ser Arg Phe Thr Cys Arg Gly Lys Pro Ile His His Phe Leu Gly Thr
    130                 135                 140

Ser Thr Phe Ser Gln Tyr Thr Val Val Asp Glu Ile Ser Val Ala Lys
145                 150                 155                 160

Ile Asp Ala Ala Ser Pro Leu Glu Lys Val Cys Leu Ile Gly Cys Gly
                165                 170                 175

Phe Ser Thr Gly Tyr Gly Ser Ala Val Lys Val Ala Lys Val Thr Gln
            180                 185                 190

Gly Ser Thr Cys Ala Val Phe Gly Leu Gly Gly Val Gly Leu Ser Val
        195                 200                 205

Ile Met Gly Cys Lys Ala Ala Gly Ala Ala Arg Ile Ile Gly Val Asp
    210                 215                 220

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

Cys Val Asn Pro Gln Asp Tyr Lys Lys Pro Ile Gln Glu Val Leu Thr
                245                 250                 255

Glu Met Ser Asn Gly Gly Val Asp Phe Ser Phe Glu Val Ile Gly Arg
            260                 265                 270

Leu Asp Thr Met Val Thr Ala Leu Ser Cys Cys Gln Glu Ala Tyr Gly
        275                 280                 285

Val Ser Val Ile Val Gly Val Pro Pro Asp Ser Gln Asn Leu Ser Met
    290                 295                 300

Asn Pro Met Leu Leu Leu Ser Gly Arg Thr Trp Lys Gly Ala Ile Phe
305                 310                 315                 320

Gly Gly Phe Lys Ser Lys Asp Ser Val Pro Lys Leu Val Ala Asp Phe
                325                 330                 335

Met Ala Lys Lys Phe Ala Leu Asp Pro Leu Ile Thr His Val Leu Pro
            340                 345                 350

Phe Glu Lys Ile Asn Glu Gly Phe Asp Leu Leu Arg Ser Gly Glu Ser
        355                 360                 365

Ile Arg Thr Ile Leu Thr Phe
    370                 375
```

<210> SEQ ID NO 143
<211> LENGTH: 1206
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Aureobasidin A resistance (AUR1-C)

<400> SEQUENCE: 143

atggcaaacc ctttttcgag atggtttcta tcagagagac ctccaaactg ccatgtagcc 60

gatttagaaa caagtttaga tccccatcaa acgttgttga aggtgcaaaa atacaaaccc 120

gctttaagcg actgggtgca ttacatcttc ttgggatcca tcatgctgtt tgtgttcatt 180

actaatcccg caccttggat cttcaagatc ctttttttatt gtttcttggg cactttattc 240

atcattccag ctacgtcaca gtttttcttc aatgccttgc ccatcctaac atgggtggcg 300

ctgtatttca cttcatcgta ctttccagat gaccgcaggc ctcctattac tgtcaaagtg 360

ttaccagcgg tggaaacaat tttatacggc gacaatttaa gtgatattct tgcaacatcg 420

acgaattcct ttttggacat tttagcatgg ttaccgtacg gactatttca ttatggggcc 480

ccatttgtcg ttgctgccat cttattcgta tttggtccac caactgtttt gcaaggttat 540

gcttttgcat ttggttatat gaacctgttt ggtgttatca tgcaaaatgt ctttccagcc 600

gctcccccat ggtataaaat tctctatgga ttgcaatcag ccaactatga tatgcatggc 660

tcgcctggtg gattagctag aattgataag ctactcggta ttaatatgta tactacatgt 720

ttttcaaatt cctccgtcat tttcggtgct tttccttcac tgcattccgg gtgtgctact 780

atggaagccc tgtttttctg ttattgtttt ccaaaattga agcccttgtt tattgcttat 840

gtttgctggt tatggtggtc aactatgtat ctgacacacc attattttgt agaccttatg 900

gcaggttctg tgctgtcata cgttattttc cagtacacaa agtacacaca tttaccaatt 960

gtagatacat ctcttttttg cagatggtca tacacttcaa ttgagaaata cgatatatca 1020

aagagtgatc cattggctgc agattcaaac gatatcgaaa gtgtcccttt gtccaacttg 1080

gaacttgact ttgatcttaa tatgactgat gaacccagtg taagcccttc gttatttgat 1140

ggatctactt ctgtttctcg ttcgtccgcc acgtctataa cgtcactagg tgtaaagagg 1200

gcttaa 1206

<210> SEQ ID NO 144
<211> LENGTH: 401
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Aureobasidin A resistance (AUR1-C)

<400> SEQUENCE: 144

Met Ala Asn Pro Phe Ser Arg Trp Phe Leu Ser Glu Arg Pro Pro Asn
1 5 10 15

Cys His Val Ala Asp Leu Glu Thr Ser Leu Asp Pro His Gln Thr Leu
20 25 30

Leu Lys Val Gln Lys Tyr Lys Pro Ala Leu Ser Asp Trp Val His Tyr
35 40 45

Ile Phe Leu Gly Ser Ile Met Leu Phe Val Phe Ile Thr Asn Pro Ala
50 55 60

Pro Trp Ile Phe Lys Ile Leu Phe Tyr Cys Phe Leu Gly Thr Leu Phe
65 70 75 80

Ile Ile Pro Ala Thr Ser Gln Phe Phe Phe Asn Ala Leu Pro Ile Leu
85 90 95

```
Thr Trp Val Ala Leu Tyr Phe Thr Ser Ser Tyr Phe Pro Asp Asp Arg
            100                 105                 110

Arg Pro Pro Ile Thr Val Lys Val Leu Pro Ala Val Glu Thr Ile Leu
        115                 120                 125

Tyr Gly Asp Asn Leu Ser Asp Ile Leu Ala Thr Ser Thr Asn Ser Phe
    130                 135                 140

Leu Asp Ile Leu Ala Trp Leu Pro Tyr Gly Leu Phe His Tyr Gly Ala
145                 150                 155                 160

Pro Phe Val Val Ala Ala Ile Leu Phe Val Phe Gly Pro Pro Thr Val
                165                 170                 175

Leu Gln Gly Tyr Ala Phe Ala Phe Gly Tyr Met Asn Leu Phe Gly Val
            180                 185                 190

Ile Met Gln Asn Val Phe Pro Ala Ala Pro Pro Trp Tyr Lys Ile Leu
        195                 200                 205

Tyr Gly Leu Gln Ser Ala Asn Tyr Asp Met His Gly Ser Pro Gly Gly
    210                 215                 220

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

Phe Ser Asn Ser Ser Val Ile Phe Gly Ala Phe Pro Ser Leu His Ser
                245                 250                 255

Gly Cys Ala Thr Met Glu Ala Leu Phe Phe Cys Tyr Cys Phe Pro Lys
            260                 265                 270

Leu Lys Pro Leu Phe Ile Ala Tyr Val Cys Trp Leu Trp Trp Ser Thr
        275                 280                 285

Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Met Ala Gly Ser Val
    290                 295                 300

Leu Ser Tyr Val Ile Phe Gln Tyr Thr Lys Tyr Thr His Leu Pro Ile
305                 310                 315                 320

Val Asp Thr Ser Leu Phe Cys Arg Trp Ser Tyr Thr Ser Ile Glu Lys
                325                 330                 335

Tyr Asp Ile Ser Lys Ser Asp Pro Leu Ala Ala Asp Ser Asn Asp Ile
            340                 345                 350

Glu Ser Val Pro Leu Ser Asn Leu Glu Leu Asp Phe Asp Leu Asn Met
        355                 360                 365

Thr Asp Glu Pro Ser Val Ser Pro Ser Leu Phe Asp Gly Ser Thr Ser
    370                 375                 380

Val Ser Arg Ser Ser Ala Thr Ser Ile Thr Ser Leu Gly Val Lys Arg
385                 390                 395                 400

Ala


<210> SEQ ID NO 145
<211> LENGTH: 552
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: bialiphos resistance protein

<400> SEQUENCE: 145

atgagcccag aacgacgccc ggccgacatc cgccgtgcca ccgaggcgga catgccggcg     60

gtctgcacca tcgtcaacca ctacatcgag acaagcacgg tcaacttccg taccgagccg    120

caggaaccgc aggagtggac ggacgacctc gtccgtctgc gggagcgcta tccctggctc    180

gtcgccgagg tggacggcga ggtcgccggc atcgcctacg cgggcccctg gaaggcacgc    240

aacgcctacg actggacggc cgagtcgacc gtgtacgtct ccccccgcca ccagcggacg    300
```

```
ggactgggct ccacgctcta cacccacctg ctgaagtccc tggaggcaca gggcttcaag    360

agcgtggtcg ctgtcatcgg gctgcccaac gacccgagcg tgcgcatgca cgaggcgctc    420

ggatatgccc cccgcggcat gctgcgggcg gccggcttca agcacgggaa ctggcatgac    480

gtgggtttct ggcagctgga cttcagcctg ccggtaccgc cccgtccggt cctgcccgtc    540

accgagattt ga                                                        552


<210> SEQ ID NO 146
<211> LENGTH: 183
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: bialiphos resistance protein

<400> SEQUENCE: 146

Met Ser Pro Glu Arg Arg Pro Ala Asp Ile Arg Arg Ala Thr Glu Ala
1               5                   10                  15

Asp Met Pro Ala Val Cys Thr Ile Val Asn His Tyr Ile Glu Thr Ser
            20                  25                  30

Thr Val Asn Phe Arg Thr Glu Pro Gln Glu Pro Gln Glu Trp Thr Asp
        35                  40                  45

Asp Leu Val Arg Leu Arg Glu Arg Tyr Pro Trp Leu Val Ala Glu Val
    50                  55                  60

Asp Gly Glu Val Ala Gly Ile Ala Tyr Ala Gly Pro Trp Lys Ala Arg
65                  70                  75                  80

Asn Ala Tyr Asp Trp Thr Ala Glu Ser Thr Val Tyr Val Ser Pro Arg
                85                  90                  95

His Gln Arg Thr Gly Leu Gly Ser Thr Leu Tyr Thr His Leu Leu Lys
            100                 105                 110

Ser Leu Glu Ala Gln Gly Phe Lys Ser Val Val Ala Val Ile Gly Leu
        115                 120                 125

Pro Asn Asp Pro Ser Val Arg Met His Glu Ala Leu Gly Tyr Ala Pro
    130                 135                 140

Arg Gly Met Leu Arg Ala Ala Gly Phe Lys His Gly Asn Trp His Asp
145                 150                 155                 160

Val Gly Phe Trp Gln Leu Asp Phe Ser Leu Pro Val Pro Pro Arg Pro
                165                 170                 175

Val Leu Pro Val Thr Glu Ile
            180


<210> SEQ ID NO 147
<211> LENGTH: 1953
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cerulenin resistance YML007W  Chr 13

<400> SEQUENCE: 147

atgagtgtgt ctaccgccaa gaggtcgctg gatgtcgttt ctccgggttc attagcggag     60

tttgagggtt caaaatctcg tcacgatgaa atagaaaatg aacatagacg tactggtaca    120

cgtgatggcg aggatagcga gcaaccgaag aagaagggta gcaaaactag caaaaagcaa    180

gatttggatc ctgaaactaa gcagaagagg actgcccaaa atcgggccgc tcaaagagct    240

tttagggaac gtaaggagag gaagatgaag gaattggaga agaaggtaca aagtttagag    300

agtattcagc agcaaaatga agtggaagct acttttttga gggaccagtt aatcactctg    360

gtgaatgagt taaaaaaata tagaccagag acaagaaatg actcaaaagt gctggaatat    420
```

```
ttagcaaggc gagatcctaa tttgcatttt tcaaaaaata acgttaacca cagcaatagc    480

gagccaattg acacacccaa tgatgacata caagaaaatg ttaaacaaaa gatgaatttc    540

acgtttcaat atccgcttga taacgacaac gacaacgaca acagtaaaaa tgtggggaaa    600

caattacctt caccaaatga tccaagtcat tcggctccta tgcctataaa tcagacacaa    660

aagaaattaa gtgacgctac agattcctcc agcgctactt tggattccct ttcaaatagt    720

aacgatgttc ttaataacac accaaactcc tccacttcga tggattggtt agataatgta    780

atatatacta acaggtttgt gtcaggtgat gatggcagca atagtaaaac taagaattta    840

gacagtaata tgttttctaa tgactttaat tttgaaaacc aatttgatga acaagtttcg    900

gagttttgtt cgaaaatgaa ccaggtatgt ggaacaaggc aatgtcccat tcccaagaaa    960

cccatctcgg ctcttgataa agaagttttc gcgtcatctt ctatactaag ttcaaattct   1020

cctgctttaa caaatacttg ggaatcacat tctaatatta cagataatac tcctgctaat   1080

gtcattgcta ctgatgctac taaatatgaa aattccttct ccggttttgg ccgacttggt   1140

ttcgatatga gtgccaatca ttacgtcgtg aatgataata gcactggtag cactgatagc   1200

actggtagca ctggcaataa gaacaaaaag aacaataata atagcgatga tgtactccca   1260

ttcatatccg agtcaccgtt tgatatgaac caagttacta attttttag tccgggatct    1320

accggcatcg gcaataatgc tgcctctaac accaatccca gcctactgca aagcagcaaa   1380

gaggatatac cttttatcaa cgcaaatctg gctttcccag acgacaattc aactaatatt   1440

caattacaac ctttctctga atctcaatct caaaataagt ttgactacga catgtttttt   1500

agagattcat cgaaggaagg taacaattta tttggagagt tttagagga tgacgatgat    1560

gacaaaaaag ccgctaatat gtcagacgat gagtcaagtt taatcaagaa ccagttaatt   1620

aacgaagaac cagagcttcc gaaacaatat ctacaatcgg taccaggaaa tgaaagcgaa   1680

atctcacaaa aaaatggcag tagtttacag aatgctgaca aaatcaataa tggcaatgat   1740

aacgataatg ataatgatgt cgttccatct aaggaaggct ctttactaag gtgttcggaa   1800

atttgggata gaataacaac acatccgaaa tactcagata ttgatgtcga tggtttatgt   1860

tccgagctaa tggcaaaggc aaaatgttca gaaagagggg ttgtcatcaa tgcagaagac   1920

gttcaattag ctttgaataa gcatatgaac taa                                1953
```

```
<210> SEQ ID NO 148
<211> LENGTH: 650
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cerulenin resistance YML007W  Chr 13

<400> SEQUENCE: 148

Met Ser Val Ser Thr Ala Lys Arg Ser Leu Asp Val Val Ser Pro Gly
1               5                   10                  15

Ser Leu Ala Glu Phe Glu Gly Ser Lys Ser Arg His Asp Glu Ile Glu
            20                  25                  30

Asn Glu His Arg Arg Thr Gly Thr Arg Asp Gly Glu Asp Ser Glu Gln
        35                  40                  45

Pro Lys Lys Lys Gly Ser Lys Thr Ser Lys Lys Gln Asp Leu Asp Pro
    50                  55                  60

Glu Thr Lys Gln Lys Arg Thr Ala Gln Asn Arg Ala Ala Gln Arg Ala
65                  70                  75                  80

Phe Arg Glu Arg Lys Glu Arg Lys Met Lys Glu Leu Glu Lys Lys Val
```

```
                85                  90                  95

Gln Ser Leu Glu Ser Ile Gln Gln Gln Asn Glu Val Glu Ala Thr Phe
            100                 105                 110

Leu Arg Asp Gln Leu Ile Thr Leu Val Asn Glu Leu Lys Lys Tyr Arg
        115                 120                 125

Pro Glu Thr Arg Asn Asp Ser Lys Val Leu Glu Tyr Leu Ala Arg Arg
    130                 135                 140

Asp Pro Asn Leu His Phe Ser Lys Asn Asn Val Asn His Ser Asn Ser
145                 150                 155                 160

Glu Pro Ile Asp Thr Pro Asn Asp Asp Ile Gln Glu Asn Val Lys Gln
                165                 170                 175

Lys Met Asn Phe Thr Phe Gln Tyr Pro Leu Asp Asn Asp Asn Asp Asn
            180                 185                 190

Asp Asn Ser Lys Asn Val Gly Lys Gln Leu Pro Ser Pro Asn Asp Pro
        195                 200                 205

Ser His Ser Ala Pro Met Pro Ile Asn Gln Thr Gln Lys Lys Leu Ser
    210                 215                 220

Asp Ala Thr Asp Ser Ser Ser Ala Thr Leu Asp Ser Leu Ser Asn Ser
225                 230                 235                 240

Asn Asp Val Leu Asn Asn Thr Pro Asn Ser Ser Thr Ser Met Asp Trp
                245                 250                 255

Leu Asp Asn Val Ile Tyr Thr Asn Arg Phe Val Ser Gly Asp Asp Gly
            260                 265                 270

Ser Asn Ser Lys Thr Lys Asn Leu Asp Ser Asn Met Phe Ser Asn Asp
        275                 280                 285

Phe Asn Phe Glu Asn Gln Phe Asp Glu Gln Val Ser Glu Phe Cys Ser
    290                 295                 300

Lys Met Asn Gln Val Cys Gly Thr Arg Gln Cys Pro Ile Pro Lys Lys
305                 310                 315                 320

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

Ser Ser Asn Ser Pro Ala Leu Thr Asn Thr Trp Glu Ser His Ser Asn
            340                 345                 350

Ile Thr Asp Asn Thr Pro Ala Asn Val Ile Ala Thr Asp Ala Thr Lys
        355                 360                 365

Tyr Glu Asn Ser Phe Ser Gly Phe Gly Arg Leu Gly Phe Asp Met Ser
    370                 375                 380

Ala Asn His Tyr Val Val Asn Asp Asn Ser Thr Gly Ser Thr Asp Ser
385                 390                 395                 400

Thr Gly Ser Thr Gly Asn Lys Asn Lys Lys Asn Asn Asn Asn Ser Asp
                405                 410                 415

Asp Val Leu Pro Phe Ile Ser Glu Ser Pro Phe Asp Met Asn Gln Val
            420                 425                 430

Thr Asn Phe Phe Ser Pro Gly Ser Thr Gly Ile Gly Asn Asn Ala Ala
        435                 440                 445

Ser Asn Thr Asn Pro Ser Leu Leu Gln Ser Ser Lys Glu Asp Ile Pro
    450                 455                 460

Phe Ile Asn Ala Asn Leu Ala Phe Pro Asp Asp Asn Ser Thr Asn Ile
465                 470                 475                 480

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

Asp Met Phe Phe Arg Asp Ser Ser Lys Glu Gly Asn Asn Leu Phe Gly
            500                 505                 510
```

```
Glu Phe Leu Glu Asp Asp Asp Asp Asp Lys Lys Ala Ala Asn Met Ser
        515                 520                 525

Asp Asp Glu Ser Ser Leu Ile Lys Asn Gln Leu Ile Asn Glu Glu Pro
    530                 535                 540

Glu Leu Pro Lys Gln Tyr Leu Gln Ser Val Pro Gly Asn Glu Ser Glu
545                 550                 555                 560

Ile Ser Gln Lys Asn Gly Ser Ser Leu Gln Asn Ala Asp Lys Ile Asn
                565                 570                 575

Asn Gly Asn Asp Asn Asp Asn Asp Asn Asp Val Val Pro Ser Lys Glu
            580                 585                 590

Gly Ser Leu Leu Arg Cys Ser Glu Ile Trp Asp Arg Ile Thr Thr His
        595                 600                 605

Pro Lys Tyr Ser Asp Ile Asp Val Asp Gly Leu Cys Ser Glu Leu Met
    610                 615                 620

Ala Lys Ala Lys Cys Ser Glu Arg Gly Val Val Ile Asn Ala Glu Asp
625                 630                 635                 640

Val Gln Leu Ala Leu Asn Lys His Met Asn
                645                 650
```

<210> SEQ ID NO 149
<211> LENGTH: 810
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Geneticin resistance (kanMX)

<400> SEQUENCE: 149

```
atgggtaagg aaaagactca cgtttcgagg ccgcgattaa attccaacat ggatgctgat      60

ttatatgggt ataaatgggc tcgcgataat gtcgggcaat caggtgcgac aatctatcga     120

ttgtatggga agcccgatgc gccagagttg tttctgaaac atggcaaagg tagcgttgcc     180

aatgatgtta cagatgagat ggtcagacta aactggctga cggaatttat gcctcttccg     240

accatcaagc attttatccg tactcctgat gatgcatggt tactcaccac tgcgatcccc     300

ggcaaaacag cattccaggt attagaagaa tatcctgatt caggtgaaaa tattgttgat     360

gcgctggcag tgttcctgcg ccggttgcat tcgattcctg tttgtaattg tccttttaac     420

agcgatcgcg tatttcgtct cgctcaggcg caatcacgaa tgaataacgg tttggttgat     480

gcgagtgatt ttgatgacga gcgtaatggc tggcctgttg aacaagtctg gaaagaaatg     540

cataagcttt tgccattctc accggattca gtcgtcactc atggtgattt ctcacttgat     600

aaccttattt ttgacgaggg gaaattaata ggttgtattg atgttggacg agtcggaatc     660

gcagaccgat accaggatct tgccatccta tggaactgcc tcggtgagtt ttctccttca     720

ttacagaaac ggctttttca aaaatatggt attgataatc ctgatatgaa taaattgcag     780

tttcatttga tgctcgatga gtttttctaa                                      810
```

<210> SEQ ID NO 150
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Geneticin resistance (kanMX)

<400> SEQUENCE: 150

```
Met Gly Lys Glu Lys Thr His Val Ser Arg Pro Arg Leu Asn Ser Asn
1               5                   10                  15
```

```
Met Asp Ala Asp Leu Tyr Gly Tyr Lys Trp Ala Arg Asp Asn Val Gly
            20                  25                  30

Gln Ser Gly Ala Thr Ile Tyr Arg Leu Tyr Gly Lys Pro Asp Ala Pro
        35                  40                  45

Glu Leu Phe Leu Lys His Gly Lys Gly Ser Val Ala Asn Asp Val Thr
    50                  55                  60

Asp Glu Met Val Arg Leu Asn Trp Leu Thr Glu Phe Met Pro Leu Pro
65                  70                  75                  80

Thr Ile Lys His Phe Ile Arg Thr Pro Asp Asp Ala Trp Leu Leu Thr
                85                  90                  95

Thr Ala Ile Pro Gly Lys Thr Ala Phe Gln Val Leu Glu Glu Tyr Pro
            100                 105                 110

Asp Ser Gly Glu Asn Ile Val Asp Ala Leu Ala Val Phe Leu Arg Arg
        115                 120                 125

Leu His Ser Ile Pro Val Cys Asn Cys Pro Phe Asn Ser Asp Arg Val
    130                 135                 140

Phe Arg Leu Ala Gln Ala Gln Ser Arg Met Asn Asn Gly Leu Val Asp
145                 150                 155                 160

Ala Ser Asp Phe Asp Asp Glu Arg Asn Gly Trp Pro Val Glu Gln Val
                165                 170                 175

Trp Lys Glu Met His Lys Leu Leu Pro Phe Ser Pro Asp Ser Val Val
            180                 185                 190

Thr His Gly Asp Phe Ser Leu Asp Asn Leu Ile Phe Asp Glu Gly Lys
        195                 200                 205

Leu Ile Gly Cys Ile Asp Val Gly Arg Val Gly Ile Ala Asp Arg Tyr
    210                 215                 220

Gln Asp Leu Ala Ile Leu Trp Asn Cys Leu Gly Glu Phe Ser Pro Ser
225                 230                 235                 240

Leu Gln Lys Arg Leu Phe Gln Lys Tyr Gly Ile Asp Asn Pro Asp Met
                245                 250                 255

Asn Lys Leu Gln Phe His Leu Met Leu Asp Glu Phe Phe
            260                 265
```

<210> SEQ ID NO 151
<211> LENGTH: 1026
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hygromycin B resistance (HygR)

<400> SEQUENCE: 151

```
atgaaaaagc ctgaactcac cgcgacgtct gtcgagaagt ttctgatcga aaagttcgac      60

agcgtctccg acctgatgca gctctcggag ggcgaagaat ctcgtgcttt cagcttcgat     120

gtaggagggc gtggatatgt cctgcgggta aatagctgcg ccgatggttt ctacaaagat     180

cgttatgttt atcggcactt tgcatcggcc gcgctcccga ttccggaagt gcttgacatt     240

ggggagttca gcgagagcct gacctattgc atctcccgcc gtgcacaggg tgtcacgttg     300

caagacctgc ctgaaaccga actgcccgct gttctccagc cggtcgcgga ggccatggat     360

gcgatcgctg cggccgatct tagccagacg agcgggttcg gcccattcgg accgcaagga     420

atcggtcaat acactacatg gcgtgatttc atatgcgcga ttgctgatcc ccatgtgtat     480

cactggcaaa ctgtgatgga cgacaccgtc agtgcgtccg tcgcgcaggc tctcgatgag     540

ctgatgcttt gggccgagga ctgccccgaa gtccggcacc tcgtgcatgc ggatttcggc     600

tccaacaatg tcctgacgga caatggccgc ataacagcgg tcattgactg gagcgaggcg     660
```

```
atgttcgggg attcccaata cgaggtcgcc aacatcttct tctggaggcc gtggttggct    720

tgtatggagc agcagacgcg ctacttcgag cggaggcatc cggagcttgc aggatcgccg    780

cgcctccggg cgtatatgct ccgcattggt cttgaccaac tctatcagag cttggttgac    840

ggcaatttcg atgatgcagc ttgggcgcag ggtcgatgcg acgcaatcgt ccgatccgga    900

gccgggactg tcgggcgtac acaaatcgcc cgcagaagcg cggccgtctg gaccgatggc    960

tgtgtagaag tactcgccga tagtggaaac cgacgcccca gcactcgtcc gagggcaaag   1020

gaatag                                                              1026
```

<210> SEQ ID NO 152
<211> LENGTH: 341
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hygromycin B resistance (HygR)

<400> SEQUENCE: 152

```
Met Lys Lys Pro Glu Leu Thr Ala Thr Ser Val Glu Lys Phe Leu Ile
1               5                   10                  15

Glu Lys Phe Asp Ser Val Ser Asp Leu Met Gln Leu Ser Glu Gly Glu
            20                  25                  30

Glu Ser Arg Ala Phe Ser Phe Asp Val Gly Gly Arg Gly Tyr Val Leu
        35                  40                  45

Arg Val Asn Ser Cys Ala Asp Gly Phe Tyr Lys Asp Arg Tyr Val Tyr
    50                  55                  60

Arg His Phe Ala Ser Ala Ala Leu Pro Ile Pro Glu Val Leu Asp Ile
65                  70                  75                  80

Gly Glu Phe Ser Glu Ser Leu Thr Tyr Cys Ile Ser Arg Arg Ala Gln
                85                  90                  95

Gly Val Thr Leu Gln Asp Leu Pro Glu Thr Glu Leu Pro Ala Val Leu
            100                 105                 110

Gln Pro Val Ala Glu Ala Met Asp Ala Ile Ala Ala Ala Asp Leu Ser
        115                 120                 125

Gln Thr Ser Gly Phe Gly Pro Phe Gly Pro Gln Gly Ile Gly Gln Tyr
    130                 135                 140

Thr Thr Trp Arg Asp Phe Ile Cys Ala Ile Ala Asp Pro His Val Tyr
145                 150                 155                 160

His Trp Gln Thr Val Met Asp Asp Thr Val Ser Ala Ser Val Ala Gln
                165                 170                 175

Ala Leu Asp Glu Leu Met Leu Trp Ala Glu Asp Cys Pro Glu Val Arg
            180                 185                 190

His Leu Val His Ala Asp Phe Gly Ser Asn Asn Val Leu Thr Asp Asn
        195                 200                 205

Gly Arg Ile Thr Ala Val Ile Asp Trp Ser Glu Ala Met Phe Gly Asp
    210                 215                 220

Ser Gln Tyr Glu Val Ala Asn Ile Phe Phe Trp Arg Pro Trp Leu Ala
225                 230                 235                 240

Cys Met Glu Gln Gln Thr Arg Tyr Phe Glu Arg Arg His Pro Glu Leu
                245                 250                 255

Ala Gly Ser Pro Arg Leu Arg Ala Tyr Met Leu Arg Ile Gly Leu Asp
            260                 265                 270

Gln Leu Tyr Gln Ser Leu Val Asp Gly Asn Phe Asp Asp Ala Ala Trp
        275                 280                 285
```

```
Ala Gln Gly Arg Cys Asp Ala Ile Val Arg Ser Gly Ala Gly Thr Val
    290                 295                 300

Gly Arg Thr Gln Ile Ala Arg Arg Ser Ala Ala Val Trp Thr Asp Gly
305                 310                 315                 320

Cys Val Glu Val Leu Ala Asp Ser Gly Asn Arg Arg Pro Ser Thr Arg
                325                 330                 335

Pro Arg Ala Lys Glu
            340


<210> SEQ ID NO 153
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Streptomyces noursei

<400> SEQUENCE: 153

atgaccactc ttgacgacac ggcttaccgg taccgcacca gtgtcccggg ggacgccgag      60

gccatcgagg cactggatgg gtccttcacc accgacaccg tcttccgcgt caccgccacc     120

ggggacggct tcaccctgcg ggaggtgccg gtggacccgc ccctgaccaa ggtgttcccc     180

gacgacgaat cggacgacga atcggacgac ggggaggacg gcgacccgga ctcccggacg     240

ttcgtcgcgt acggggacga cggcgacctg gcgggcttcg tggtcatctc gtactcggcg     300

tggaaccgcc ggctgaccgt cgaggacatc gaggtcgccc cggagcaccg ggggcacggg     360

gtcgggcgcg cgttgatggg gctcgcgacg gagttcgccg gcgagcgggg cgccgggcac     420

ctctggctgg aggtcaccaa cgtcaacgca ccggcgatcc acgcgtaccg gcggatgggg     480

ttcaccctct gcggcctgga caccgccctg tacgacggca ccgcctcgga cggcgagcgg     540

caggcgctct acatgagcat gccctgcccc tag                                  573


<210> SEQ ID NO 154
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: Streptomyces noursei

<400> SEQUENCE: 154

Met Thr Thr Leu Asp Asp Thr Ala Tyr Arg Tyr Arg Thr Ser Val Pro
1               5                   10                  15

Gly Asp Ala Glu Ala Ile Glu Ala Leu Asp Gly Ser Phe Thr Thr Asp
            20                  25                  30

Thr Val Phe Arg Val Thr Ala Thr Gly Asp Gly Phe Thr Leu Arg Glu
        35                  40                  45

Val Pro Val Asp Pro Pro Leu Thr Lys Val Phe Pro Asp Asp Glu Ser
    50                  55                  60

Asp Asp Glu Ser Asp Asp Gly Glu Asp Gly Asp Pro Asp Ser Arg Thr
65                  70                  75                  80

Phe Val Ala Tyr Gly Asp Asp Gly Asp Leu Ala Gly Phe Val Val Ile
                85                  90                  95

Ser Tyr Ser Ala Trp Asn Arg Arg Leu Thr Val Glu Asp Ile Glu Val
            100                 105                 110

Ala Pro Glu His Arg Gly His Gly Val Gly Arg Ala Leu Met Gly Leu
        115                 120                 125

Ala Thr Glu Phe Ala Gly Glu Arg Gly Ala Gly His Leu Trp Leu Glu
    130                 135                 140

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

Phe Thr Leu Cys Gly Leu Asp Thr Ala Leu Tyr Asp Gly Thr Ala Ser
```

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                165                 170                 175

Asp Gly Glu Arg Gln Ala Leu Tyr Met Ser Met Pro Cys Pro
            180                 185                 190


<210> SEQ ID NO 155
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Phleomycin zeocin binding protein

<400> SEQUENCE: 155

atggccaagt tgaccagtgc cgttccggtg ctcaccgcgc gcgacgtcgc cggagcggtc     60

gagttctgga ccgaccggct cgggttctcc cgggacttcg tggaggacga cttcgccggt    120

gtggtccggg acgacgtgac cctgttcatc agcgcggtcc aggaccaggt ggtgccggac    180

aacaccctgg cctgggtgtg ggtgcgcggc ctggacgagc tgtacgccga gtggtcggag    240

gtcgtgtcca cgaacttccg ggacgcctcc gggccggcca tgaccgagat cggcgagcag    300

ccgtgggggc gggagttcgc cctgcgcgac ccggccggca actgcgtgca cttcgtggcc    360

gaggagcagg actga                                                     375


<210> SEQ ID NO 156
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Phleomycin zeocin binding protein

<400> SEQUENCE: 156

Met Ala Lys Leu Thr Ser Ala Val Pro Val Leu Thr Ala Arg Asp Val
1               5                   10                  15

Ala Gly Ala Val Glu Phe Trp Thr Asp Arg Leu Gly Phe Ser Arg Asp
            20                  25                  30

Phe Val Glu Asp Asp Phe Ala Gly Val Val Arg Asp Asp Val Thr Leu
        35                  40                  45

Phe Ile Ser Ala Val Gln Asp Gln Val Val Pro Asp Asn Thr Leu Ala
    50                  55                  60

Trp Val Trp Val Arg Gly Leu Asp Glu Leu Tyr Ala Glu Trp Ser Glu
65                  70                  75                  80

Val Val Ser Thr Asn Phe Arg Asp Ala Ser Gly Pro Ala Met Thr Glu
                85                  90                  95

Ile Gly Glu Gln Pro Trp Gly Arg Glu Phe Ala Leu Arg Asp Pro Ala
            100                 105                 110

Gly Asn Cys Val His Phe Val Ala Glu Glu Gln Asp
        115                 120


<210> SEQ ID NO 157
<211> LENGTH: 1539
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 157

atggcattac ttgctgtcgc aggcgtctac gctttcgcag cgttgctcgt tgcgatagtc     60

ttaaatgtca cgcgccaatt gctctttcgc aacgagaaag aaccacccgt cgtcttccat    120

tggatcccct tcttgggaag cacaatcagc tatggaatgg acccctatac attcttcttc    180

tcctgcagaa aaaagtacgg ggacatcttc accttcgtgc ttctgggcca gaagaccact    240
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gtatacttgg gcgttcaagg caacgatttc atcctcaatg gcaaactcaa ggacgtgagc    300
gcggaagagg tctacagccc cctcaccacc ccggtgttcg ggtccgatgt tgtgtacgac    360
tgccctaatt ccaagctgat ggagcaaaaa aagttcatca agtttggcct cacgcaagcg    420
gcgctcgagt cacacgtcca gctgatcgaa aaggaaactc tcgactatct ccgggactct    480
ccacgcttca acggcgcgag tggagtcatt gatattcctg ctgccatggc tgagattaca    540
atctatactg ctgcgcgcgc gttgcagggc gaggaggtcc gcaagaagct cacggcagag    600
ttcgctgaac tgtaccacga tctagacaag ggattcagcc ccattaactt catgctccct    660
tgggctccat tgccgcacaa ccggaagcgt gatgctgctc atgctcggat gagagaaatc    720
tacacggaca ttatcaacga acgacgcaag aacccagacg aggagaagtc agacatgatc    780
tggaatctga tgcattgcac ctacaagagt ggccagccgg tcccggacaa agagattgct    840
cacatgatga tcactctgtt gatggcaggg caacactcgt cttcttcgat tagttcttgg    900
atcatgctgc gattggcctc ggagcctcag gtgcttgaag agctctacca agaacagctg    960
gccagcctta gcaacagaaa tggagtcttc gagccgctgc agtatcagga ccttgacaag   1020
ctgccattcc tccagagtgt catcaaggag actctacgga tccactcgtc catccactcg   1080
atcatgcgca aggtgaaaaa cccgctacca gtacctggca cctcctacat tattcccgaa   1140
gaccatgttc tactcgcctc accaggcgta accgcgctta gtgacgaata ctttcctaac   1200
gcaaccaggt gggatccgca tcgttgggag aatcagcctg acaaagagga ggatggagag   1260
atggtggact acggatatgg cagcgtgtcg aagggcactg ctagtcccta tctacctttt   1320
ggcgctggcc gtcaccgctg cattggagag aagttcgcct acgtcaactt gggcgtcatt   1380
atcgcgacca tagtgcgcca cttgaagcta ttcaatgtgg atggcaggaa aggagtgccc   1440
ggaaccgatt actcgaccct cttctccggt cccatgaagc ctgctatagt gggttgggag   1500
cgacgcttcc cggacaacat caaagggtcc atgaactaa                          1539
```

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<210> SEQ ID NO 158
<211> LENGTH: 4519
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLA54

<400> SEQUENCE: 158

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gttatcgaac agagaaacta aatccacatt aattgagagt tctatctatt agaaaatgca    120
aactccaact aaatgggaaa acagataacc tcttttattt ttttttaatg tttgatattc    180
gagtcttttt cttttgttag gtttatattc atcatttcaa tgaataaaag aagcttctta    240
ttttggttgc aaagaatgaa aaaaaaggat tttttcatac ttctaaagct tcaattataa    300
ccaaaaattt tataaatgaa gagaaaaaat ctagtagtat caagttaaac ttagaaaaac    360
tcatcgagca tcaaatgaaa ctgcaattta ttcatatcag gattatcaat accatatttt    420
tgaaaaagcc gtttctgtaa tgaaggagaa aactcaccga ggcagttcca taggatggca    480
agatcctggt atcggtctgc gattccgact cgtccaacat caatacaacc tattaatttc    540
ccctcgtcaa aaataaggtt atcaagtgag aaatcaccat gagtgacgac tgaatccggt    600
gagaatggca aaagcttatg catttctttc cagacttgtt caacaggcca gccattacgc    660
tcgtcatcaa aatcactcgc atcaaccaaa ccgttattca ttcgtgattg cgcctgagcg    720
agacgaaata cgcgatcgct gttaaaagga caattacaaa caggaatcga atgcaaccgg    780
```

```
cgcaggaaca ctgccagcgc atcaacaata ttttcacctg aatcaggata ttcttctaat    840
acctggaatg ctgttttgcc ggggatcgca gtggtgagta accatgcatc atcaggagta    900
cggataaaat gcttgatggt cggaagaggc ataaattccg tcagccagtt tagtctgacc    960
atctcatctg taacatcatt ggcaacgcta cctttgccat gtttcagaaa caactctggc   1020
gcatcgggct tcccatacaa tcgatagatt gtcgcacctg attgcccgac attatcgcga   1080
gcccatttat acccatataa atcagcatcc atgttggaat ttaatcgcgg cctcgaaacg   1140
tgagtctttt ccttacccat ctcgagtttt aatgttactt ctcttgcagt tagggaacta   1200
taatgtaact caaaataaga ttaaacaaac taaaataaaa agaagttata cagaaaaacc   1260
catataaacc agtactaatc cataataata atacacaaaa aaactatcaa ataaaaccag   1320
aaaacagatt gaatagaaaa attttttcga tctcctttta tattcaaaat tcgatatatg   1380
aaaaagggaa ctctcagaaa atcaccaaat caatttaatt agatttttct tttccttcta   1440
gcgttggaaa gaaaaatttt tctttttttt tttagaaatg aaaaattttt gccgtaggaa   1500
tcaccgtata aaccctgtat aaacgctact ctgttcacct gtgtaggcta tgattgaccc   1560
agtgttcatt gttattgcga gagagcggga gaaaagaacc gatacaagag atccatgctg   1620
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gtacgtgtgt gactattaag tagttatgat agagaggttt gtacggtgtg ttctgtgtaa   1740
ttcgattgag aaaatggtta tgaatcccta gataacttcg tataatgtat gctatacgaa   1800
gttatctgaa cattagaata cgtaatccgc aatgcgggga tcctctagag tcgacctgca   1860
ggcatgcaag cttggcgtaa tcatggtcat agctgtttcc tgtgtgaaat tgttatccgc   1920
tcacaattcc acacaacata cgagccggaa gcataaagtg taaagcctgg ggtgcctaat   1980
gagtgagcta actcacatta attgcgttgc gctcactgcc cgctttccag tcgggaaacc   2040
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cggtatcagc tcactcaaag gcggtaatac ggttatccac agaatcaggg gataacgcag   2220
gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag gccgcgttgc   2280
tggcgttttt ccataggctc cgcccccctg acgagcatca caaaaatcga cgctcaagtc   2340
agaggtggcg aaacccgaca ggactataaa gataccaggc gtttccccct ggaagctccc   2400
tcgtgcgctc tcctgttccg accctgccgc ttaccggata cctgtccgcc tttctccctt   2460
cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg gtgtaggtcg   2520
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ccggtaacta tcgtcttgag tccaacccgg taagacacga cttatcgcca ctggcagcag   2640
ccactggtaa caggattagc agagcgaggt atgtaggcgg tgctacagag ttcttgaagt   2700
ggtggcctaa ctacggctac actagaagga cagtatttgg tatctgcgct ctgctgaagc   2760
cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaaacc accgctggta   2820
gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag aaaaaaagga tctcaagaag   2880
atcctttgat cttttctacg gggtctgacg ctcagtggaa cgaaaactca cgttaaggga   2940
ttttggtcat gagattatca aaaggatctt cacctagatc cttttaaatt aaaaatgaa    3000
gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac caatgcttaa   3060
tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt gcctgactcc   3120
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```
ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt gctgcaatga   3180
taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag ccagccggaa   3240
gggccgagcg cagaagtggt cctgcaactt tatccgcctc catccagtct attaattgtt   3300
gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacgtt gttgccattg   3360
ctacaggcat cgtggtgtca cgctcgtcgt ttggtatggc ttcattcagc tccggttccc   3420
aacgatcaag gcgagttaca tgatccccca tgttgtgcaa aaaagcggtt agctccttcg   3480
gtcctccgat cgttgtcaga agtaagttgg ccgcagtgtt atcactcatg gttatggcag   3540
cactgcataa ttctcttact gtcatgccat ccgtaagatg cttttctgtg actggtgagt   3600
actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct tgcccggcgt   3660
caatacggga taataccgcg ccacatagca gaactttaaa agtgctcatc attggaaaac   3720
gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt tcgatgtaac   3780
ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt tctgggtgag   3840
caaaaacagg aaggcaaaat gccgcaaaaa agggaataag ggcgacacgg aaatgttgaa   3900
tactcatact cttccttttt caatattatt gaagcattta tcagggttat tgtctcatga   3960
gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg cgcacatttc   4020
cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta acctataaaa   4080
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gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc gggagcagac   4200
aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg gggctggctt aactatgcgg   4260
catcagagca gattgtactg agagtgcacc atatgcggtg tgaaataccg cacagatgcg   4320
taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac tgttgggaag   4380
ggcgatcggt gcgggcctct tcgctattac gccagctggc gaaaggggga tgtgctgcaa   4440
ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa acgacggcca   4500
gtgaattcga gctcggtac                                                4519
```

<210> SEQ ID NO 159
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 749

<400> SEQUENCE: 159

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caagtctttt gtgccttccc gtcgg                                           25
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<210> SEQ ID NO 160
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 413

<400> SEQUENCE: 160

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ggacataaaa tacacaccga gattc                                           25
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<210> SEQ ID NO 161
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA468

<400> SEQUENCE: 161

gcctcgagtt ttaatgttac ttctcttgca gttaggga 38

<210> SEQ ID NO 162
<211> LENGTH: 10934
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pRS423::TEF(M4)-xpk1+ENO1-eutD

<400> SEQUENCE: 162

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ggtcatagct gtttcctgtg tgaaattgtt atccgctcac aattccacac aacataggag 120

ccggaagcat aaagtgtaaa gcctggggtg cctaatgagt gaggtaactc acattaattg 180

cgttgcgctc actgcccgct ttccagtcgg gaaacctgtc gtgccagctg cattaatgaa 240

tcggccaacg cgcggggaga ggcggtttgc gtattgggcg ctcttccgct tcctcgctca 300

ctgactcgct gcgctcggtc gttcggctgc ggcgagcggt atcagctcac tcaaaggcgg 360

taatacggtt atccacagaa tcaggggata acgcaggaaa gaacatgtga gcaaaaggcc 420

agcaaaaggc caggaaccgt aaaaaggccg cgttgctggc gtttttccat aggctccgcc 480

cccctgacga gcatcacaaa aatcgacgct caagtcagag gtggcgaaac ccgacaggac 540

tataaagata ccaggcgttt ccccctggaa gctccctcgt gcgctctcct gttccgaccc 600

tgccgcttac cggatacctg tccgcctttc tcccttcggg aagcgtggcg ctttctcata 660

gctcacgctg taggtatctc agttcggtgt aggtcgttcg ctccaagctg ggctgtgtgc 720

acgaaccccc cgttcagccc gaccgctgcg ccttatccgg taactatcgt cttgagtcca 780

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cgaggtatgt aggcggtgct acagagttct tgaagtggtg gcctaactac ggctacacta 900

gaaggacagt atttggtatc tgcgctctgc tgaagccagt taccttcgga aaaagagttg 960

gtagctcttg atccggcaaa caaaccaccg ctggtagcgg tggttttttt gtttgcaagc 1020

agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt tctacggggt 1080

ctgacgctca gtggaacgaa aactcacgtt aagggatttt ggtcatgaga ttatcaaaaa 1140

ggatcttcac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc taaagtatat 1200

atgagtaaac ttggtctgac agttaccaat gcttaatcag tgaggcacct atctcagcga 1260

tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata actacgatac 1320

gggagggctt accatctggc cccagtgctg caatgatacc gcgagaccca cgctcaccgg 1380

ctccagattt atcagcaata aaccagccag ccggaagggc cgagcgcaga agtggtcctg 1440

caactttatc cgcctccatc cagtctatta attgttgccg ggaagctaga gtaagtagtt 1500

cgccagttaa tagtttgcgc aacgttgttg ccattgctac aggcatcgtg gtgtcacgct 1560

cgtcgtttgg tatggcttca ttcagctccg gttcccaacg atcaaggcga gttacatgat 1620

cccccatgtt gtgcaaaaaa gcggttagct ccttcggtcc tccgatcgtt gtcagaagta 1680

agttggccgc agtgttatca ctcatggtta tggcagcact gcataattct cttactgtca 1740

tgccatccgt aagatgcttt tctgtgactg gtgagtactc aaccaagtca ttctgagaat 1800

agtgtatgcg gcgaccgagt tgctcttgcc cggcgtcaat acgggataat accgcgccac 1860

atagcagaac tttaaaagtg ctcatcattg gaaaacgttc ttcggggcga aaactctcaa 1920

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ggatcttacc gctgttgaga tccagttcga tgtaacccac tcgtgcaccc aactgatctt   1980
cagcatcttt tactttcacc agcgtttctg ggtgagcaaa aacaggaagg caaaatgccg   2040
caaaaaaggg aataagggcg acacggaaat gttgaatact catactcttc ctttttcaat   2100
attattgaag catttatcag ggttattgtc tcatgagcgg atacatattt gaatgtattt   2160
agaaaaataa acaaataggg gttccgcgca catttccccg aaaagtgcca cctgaacgaa   2220
gcatctgtgc ttcattttgt agaacaaaaa tgcaacgcga gagcgctaat ttttcaaaca   2280
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aaacaaagaa tctgagctgc atttttacag aacagaaatg caacgcgaga gcgctatttt   2460
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cttgataact tttgcactg taggtccgtt aaggttagaa gaaggctact ttggtgtcta   2640
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aggtggatgg gtaggttata tagggatata gcacagagat atatagcaaa gagatacttt   3060
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gttcctatac tttctagaga ataggaactt cggaatagga acttcaaagc gtttccgaaa   3240
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tcctgtgata ttatcccatt ccatgcgggg tatcgtatgc ttccttcagc actacccttt   3480
agctgttcta tatgctgcca ctcctcaatt ggattagtct catccttcaa tgctatcatt   3540
tcctttgata ttggatcatc taagaaacca ttattatcat gacattaacc tataaaaata   3600
ggcgtatcac gaggcccttt cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac   3660
acatgcagct cccggagacg gtcacagctt gtctgtaagc ggatgccggg agcagacaag   3720
cccgtcaggg cgcgtcagcg ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat   3780
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cgaagaatat actaaaaaat gagcaggcaa gataaacgaa ggcaaagatg acagagcaga   4140
aagccctagt aaagcgtatt acaaatgaaa ccaagattca gattgcgatc tctttaaagg   4200
gtggtcccct agcgatagag cactcgatct tcccagaaaa agaggcagaa gcagtagcag   4260
aacaggccac acaatcgcaa gtgattaacg tccacacagg tatagggttt ctggaccata   4320
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aagtatgtat acgaacagta tgatactgaa gatgacaagg taatgcatca ttctatacgt   4920
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gaactcgacg gatctatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc   5040
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gctcattttt taaccaatag gccgaaatcg gcaaaatccc ttataaatca aaagaataga   5160
ccgagatagg gttgagtgtt gttccagttt ggaacaagag tccactatta aagaacgtgg   5220
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N1341

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<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
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aatgatagga atgtgattct tctatttttc ctttttccat tctggcagcc gtcgggaaaa 180

cgtggcttcc tctctttcgg gctctattgg agtaacgctg ccgtgagctt cctctctttc 240

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<210> SEQ ID NO 169
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N1366

<400> SEQUENCE: 169

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cttcacatac gttgcatacg 80

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N1368

<400> SEQUENCE: 170

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atatg 65

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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 171

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atgcattatg caattttttg 80

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 172

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 173

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<210> SEQ ID NO 174
<211> LENGTH: 30
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<223> OTHER INFORMATION: N1114

<400> SEQUENCE: 174

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<210> SEQ ID NO 175
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<220> FEATURE:
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<400> SEQUENCE: 175

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ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc   4920
gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct   4980
caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt   5040
taagggattt tggtcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa   5100
aaatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa   5160
tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcatc catagttgcc   5220
tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg ccccagtgct   5280
gcaatgatac cgcgagaccc acgctcaccg gctccagatt tatcagcaat aaaccagcca   5340
gccggaaggg ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt   5400
aattgttgcc gggaagctag agtaagtagt tcgccagtta atagtttgcg caacgttgtt   5460
gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc   5520
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tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc actcatggtt   5640
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ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttgc   5760
ccggcgtcaa tacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt   5820
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atgtaaccca ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct   5940
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tgttgaatac tcatactctt cctttttcaa tattattgaa gcatttatca gggttattgt   6060
ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc   6120
acatttcccc gaaaagtgcc acctgggtcc ttttcatcac gtgctataaa aataattata   6180
atttaaattt tttaatataa atatataaat taaaaataga aagtaaaaaa agaaattaaa   6240
gaaaaaatag tttttgtttt ccgaagatgt aaaagactct agggggatcg ccaacaaata   6300
ctacctttta tcttgctctt cctgctctca ggtattaatg ccgaattgtt tcatcttgtc   6360
tgtgtagaag accacacacg aaaatcctgt gattttacat tttacttatc gttaatcgaa   6420
tgtatatcta tttaatctgc ttttcttgtc taataaatat atatgtaaag tacgcttttt   6480
gttgaaattt tttaaacctt tgtttatttt tttttcttca ttccgtaact cttctacctt   6540
ctttatttac tttctaaaat ccaaatacaa aacataaaaa taaataaaca cagagtaaat   6600
tcccaaatta ttccatcatt aaaagatacg aggcgcgtgt aagttacagg caagcgatcc   6660
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gtcctaagaa accattatta tcatgacatt aacctataaa aataggcgta tcacgaggcc 6720

ctttcgtc 6728

<210> SEQ ID NO 176
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N160SeqF5

<400> SEQUENCE: 176

cctgaagtct aggtccctat tt 22

<210> SEQ ID NO 177
<211> LENGTH: 1650
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AMN1

<400> SEQUENCE: 177

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ttggagagtc cgtgtacccg cccattaaag aaaatgtcgc catcaccttc atttacgagc 120

ctgaagatgg aaaaaccgtt taaggacatt gttcgaaaat acgggggtca cctgcaccag 180

tcctcgtata acccaggttc ttcaaaagtt gaactcgtgc gtccggacct gagcttgaaa 240

acggaccaat catttttgca gagcagcgtg cagacaaccc cgaacaaaaa gagttgtaac 300

gagtatctgt ccacacccga agccactccc cttaagaaca cggccaccga gaatgcgtgg 360

gctacgtcaa gggtggtgag cgcatcaagc ctgtcaatcg tcacgccgac cgaaatcaaa 420

aatatactgg ttgacgagtt tagtgaacta aaacttggtc agcccttaac agcccagcac 480

caacggagcc atgcagtttt cgagatacct gagatcgtag agaacataat caagatgatc 540

gtttccctcg agagcgccaa tattccgaaa gaacgtccgt gcctgcgtcg caacccgcag 600

agttatgagc attcccttct gatgtataaa gacgaggaac gcgcgaagaa agcatggtcc 660

gcggctcaac aactgcgcga tccgccgctg gtgggtcata aggaaaaaaa acagggcgct 720

ctgtttagct gcatgatggt caaccgcctg tggttgaatg tcacgcgtcc gttcttattt 780

aagtctctgc atttcaaatc agtgcacaac ttcaaagaat ttctgcgcac aagtcaggaa 840

accacgcaag tgatgaggcc atcgcacttt atcctgcata aattgcacca ggtaacgcag 900

ccggatattg agagactgtc tagaatggaa tgccagaacc tcaagtggtt ggaattttat 960

gtatgtcccc gtattacacc tccactgtct tggttcgaca atttgcataa gttagaaaaa 1020

ttaatcatcc ccggaaacaa gaatatcgac gataatttcc tcttacggct gtctcagagt 1080

attcctaacc tgaaacacct cgtgcttcgt gcttgcgaca atgtttccga tagtggtgta 1140

gtttgtatcg ccctgaactg ccctaagctg aagacgttca acatcggacg tcatcgccgc 1200

ggcaatctga ttacatcagt tagcttggtt gccctgggta agtatacgca agttgagacc 1260

gttggttttg caggctgcga tgtggacgac gcaggcatat gggagttcgc gcgtttaaac 1320

gggaaaaacg tcgagcgcct gtcactcaac agttgccggc ttttaaccga ctatagcttg 1380

ccaatcctgt ttgcccttaa tagtttcccg aaccttgcgg tgttggaaat tcgaaacctc 1440

gataaaatta cagatgtccg ccattttgtg aaatataatc tgtggaagaa atcactggat 1500

gctcctatcc tgattgaggc gtgcgaacgc ataacaaagc tgattgatca ggaagagaac 1560

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cgggtcaaac gcataaatag cctggtcgct ttaaaggata tgaccgcgtg ggtgaacgct   1620

gacgatgaaa ttgaaaacaa cgtcgattga                                    1650


<210> SEQ ID NO 178
<211> LENGTH: 549
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AMN1

<400> SEQUENCE: 178

Met Lys Leu Glu Arg Val Ser Ser Asn Gly Ser Phe Lys Arg Gly Arg
1               5                   10                  15

Asp Ile Gln Ser Leu Glu Ser Pro Cys Thr Arg Pro Leu Lys Lys Met
            20                  25                  30

Ser Pro Ser Pro Ser Phe Thr Ser Leu Lys Met Glu Lys Pro Phe Lys
        35                  40                  45

Asp Ile Val Arg Lys Tyr Gly Gly His Leu His Gln Ser Ser Tyr Asn
    50                  55                  60

Pro Gly Ser Ser Lys Val Glu Leu Val Arg Pro Asp Leu Ser Leu Lys
65                  70                  75                  80

Thr Asp Gln Ser Phe Leu Gln Ser Ser Val Gln Thr Thr Pro Asn Lys
                85                  90                  95

Lys Ser Cys Asn Glu Tyr Leu Ser Thr Pro Glu Ala Thr Pro Leu Lys
            100                 105                 110

Asn Thr Ala Thr Glu Asn Ala Trp Ala Thr Ser Arg Val Val Ser Ala
        115                 120                 125

Ser Ser Leu Ser Ile Val Thr Pro Thr Glu Ile Lys Asn Ile Leu Val
    130                 135                 140

Asp Glu Phe Ser Glu Leu Lys Leu Gly Gln Pro Leu Thr Ala Gln His
145                 150                 155                 160

Gln Arg Ser His Ala Val Phe Glu Ile Pro Glu Ile Val Glu Asn Ile
                165                 170                 175

Ile Lys Met Ile Val Ser Leu Glu Ser Ala Asn Ile Pro Lys Glu Arg
            180                 185                 190

Pro Cys Leu Arg Arg Asn Pro Gln Ser Tyr Glu His Ser Leu Leu Met
        195                 200                 205

Tyr Lys Asp Glu Glu Arg Ala Lys Lys Ala Trp Ser Ala Ala Gln Gln
    210                 215                 220

Leu Arg Asp Pro Pro Leu Val Gly His Lys Glu Lys Lys Gln Gly Ala
225                 230                 235                 240

Leu Phe Ser Cys Met Met Val Asn Arg Leu Trp Leu Asn Val Thr Arg
                245                 250                 255

Pro Phe Leu Phe Lys Ser Leu His Phe Lys Ser Val His Asn Phe Lys
            260                 265                 270

Glu Phe Leu Arg Thr Ser Gln Glu Thr Thr Gln Val Met Arg Pro Ser
        275                 280                 285

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

Arg Leu Ser Arg Met Glu Cys Gln Asn Leu Lys Trp Leu Glu Phe Tyr
305                 310                 315                 320

Val Cys Pro Arg Ile Thr Pro Pro Leu Ser Trp Phe Asp Asn Leu His
                325                 330                 335

Lys Leu Glu Lys Leu Ile Ile Pro Gly Asn Lys Asn Ile Asp Asp Asn
            340                 345                 350
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Phe Leu Leu Arg Leu Ser Gln Ser Ile Pro Asn Leu Lys His Leu Val
        355                 360                 365

Leu Arg Ala Cys Asp Asn Val Ser Asp Ser Gly Val Val Cys Ile Ala
    370                 375                 380

Leu Asn Cys Pro Lys Leu Lys Thr Phe Asn Ile Gly Arg His Arg Arg
385                 390                 395                 400

Gly Asn Leu Ile Thr Ser Val Ser Leu Val Ala Leu Gly Lys Tyr Thr
                405                 410                 415

Gln Val Glu Thr Val Gly Phe Ala Gly Cys Asp Val Asp Asp Ala Gly
            420                 425                 430

Ile Trp Glu Phe Ala Arg Leu Asn Gly Lys Asn Val Glu Arg Leu Ser
        435                 440                 445

Leu Asn Ser Cys Arg Leu Leu Thr Asp Tyr Ser Leu Pro Ile Leu Phe
    450                 455                 460

Ala Leu Asn Ser Phe Pro Asn Leu Ala Val Leu Glu Ile Arg Asn Leu
465                 470                 475                 480

Asp Lys Ile Thr Asp Val Arg His Phe Val Lys Tyr Asn Leu Trp Lys
                485                 490                 495

Lys Ser Leu Asp Ala Pro Ile Leu Ile Glu Ala Cys Glu Arg Ile Thr
            500                 505                 510

Lys Leu Ile Asp Gln Glu Glu Asn Arg Val Lys Arg Ile Asn Ser Leu
        515                 520                 525

Val Ala Leu Lys Asp Met Thr Ala Trp Val Asn Ala Asp Asp Glu Ile
    530                 535                 540

Glu Asn Asn Val Asp
545
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<210> SEQ ID NO 179
<211> LENGTH: 6638
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: pLA67

<400> SEQUENCE: 179

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aaacgccagc aacgcggcct ttttacggtt cctggccttt tgctggcctt ttgctcacat     60

gttctttcct gcgttatccc ctgattctgt ggataaccgt attaccgcct ttgagtgagc    120

tgataccgct cgccgcagcc gaacgaccga gcgcagcgag tcagtgagcg aggaagcgga    180

agagcgccca atacgcaaac cgcctctccc cgcgcgttgg ccgattcatt aatgcagctg    240

gcacgacagg tttcccgact ggaaagcggg cagtgagcgc aacgcaatta atgtgagtta    300

gctcactcat taggcacccc aggctttaca ctttatgctt ccggctcgta tgttgtgtgg    360

aattgtgagc ggataacaat ttcacacagg aaacagctat gaccatgatt acgccaagct    420

tgcatgcctg caggtcgact ctagaggatc cgcattgcgg attacgtatt ctaatgttca    480

gtaccgttcg tataatgtat gctatacgaa gttatgcaga ttgtactgag agtgcaccat    540

accacagctt ttcaattcaa ttcatcattt ttttttatt cttttttttg atttcggttt    600

ctttgaaatt tttttgattc ggtaatctcc gaacagaagg aagaacgaag gaaggagcac    660

agacttagat tggtatatat acgcatatgt agtgttgaag aaacatgaaa ttgcccagta    720

ttcttaaccc aactgcacag aacaaaaacc tgcaggaaac gaagataaat catgtcgaaa    780

gctacatata aggaacgtgc tgctactcat cctagtcctg ttgctgccaa gctatttaat    840

atcatgcacg aaaagcaaac aaacttgtgt gcttcattgg atgttcgtac caccaaggaa    900
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ttactggagt tagttgaagc attaggtccc aaaatttgtt tactaaaaac acatgtggat    960
atcttgactg atttttccat ggagggcaca gttaagccgc taaaggcatt atccgccaag   1020
tacaattttt tactcttcga agacagaaaa tttgctgaca ttggtaatac agtcaaattg   1080
cagtactctg cgggtgtata cagaatagca gaatgggcag acattacgaa tgcacacggt   1140
gtggtgggcc caggtattgt tagcggtttg aagcaggcgg cagaagaagt aacaaaggaa   1200
cctagaggcc ttttgatgtt agcagaattg tcatgcaagg gctccctatc tactggagaa   1260
tatactaagg gtactgttga cattgcgaag agcgacaaag attttgttat cggctttatt   1320
gctcaaagag acatgggtgg aagagatgaa ggttacgatt ggttgattat gacacccggt   1380
gtgggtttag atgacaaggg agacgcattg ggtcaacagt atagaaccgt ggatgatgtg   1440
gtctctacag gatctgacat tattattgtt ggaagaggac tatttgcaaa gggaagggat   1500
gctaaggtag agggtgaacg ttacagaaaa gcaggctggg aagcatattt gagaagatgc   1560
ggccagcaaa actaaaaaac tgtattataa gtaaatgcat gtatactaaa ctcacaaatt   1620
agagcttcaa tttaattata tcagttatta ccctatgcgg tgtgaaatac cgcacagatg   1680
cgtaaggaga aaataccgca tcaggaaatt gtaaacgtta atattttgtt aaaattcgcg   1740
ttaaattttt gttaaatcag ctcatttttt aaccaatagg ccgaaatcgg caaaatccct   1800
tataaatcaa aagaatagac cgagataggg ttgagtgttg ttccagtttg gaacaagagt   1860
ccactattaa agaacgtgga ctccaacgtc aaagggcgaa aaaccgtcta tcagggcgat   1920
ggcccactac gtgaaccatc accctaatca agataacttc gtataatgta tgctatacga   1980
acggtaccag tgatgataca acgagttagc caaggtgaat tcgacttagg atgtctcatc   2040
aatcatctta ttcctgctgg tgtttttgt atcgccttgc cttggagtgt ttatgcttgt   2100
cctttgttca gtaaccattc ttcaagtttg tttcaagtag taggatacct tcagatatac   2160
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cctatagact atactcttca cactttaaag tacggaatta aggcccaagg gaactaacaa   2280
aaacgttcaa aaagttttaa aactatatgt gttaactgta caaaaataac ttatttatca   2340
tatcattttt ttctctgttt atttcttcta gaacttatac ctgtcttttc cttttattct   2400
ttgaatttgk tttaatatcc ctttttgktt taatatccat ccattccttt cacttagaac   2460
taataattcc cttcgtttga taatttatca ttttcctttt ctgttagtaa agtacccatt   2520
aaatgaagct ggagcgcgtg agttctaacg ggagctttaa gcgtggccgt gacatccaaa   2580
gtttggagag tccgtgtacc cgcccattaa agaaaatgtc gccatcacct tcatttacga   2640
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aaacggacca atcatttttg cagagcagcg tgcagacaac cccgaacaaa aagagttgta   2820
acgagtatct gtccacaccc gaagccactc cccttaagaa cacggccacc gagaatgcgt   2880
gggctacgtc aagggtggtg agcgcatcaa gcctgtcaat cgtcacgccg accgaaatca   2940
aaaatatact ggttgacgag tttagtgaac taaaacttgg tcagccctta acagcccagc   3000
accaacggag ccatgcagtt ttcgagatac ctgagatcgt agagaacata atcaagatga   3060
tcgtttccct cgagagcgcc aatattccga aagaacgtcc gtgcctgcgt cgcaacccgc   3120
agagttatga gcattccctt ctgatgtata aagacgagga acgcgcgaag aaagcatggt   3180
ccgcggctca acaactgcgc gatccgccgc tggtgggtca taaggaaaaa aaacagggcg   3240
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ctctgtttag ctgcatgatg gtcaaccgcc tgtggttgaa tgtcacgcgt ccgttcttat   3300
ttaagtctct gcatttcaaa tcagtgcaca acttcaaaga atttctgcgc acaagtcagg   3360
aaaccacgca agtgatgagg ccatcgcact ttatcctgca taaattgcac caggtaacgc   3420
agccggatat tgagagactg tctagaatgg aatgccagaa cctcaagtgg ttggaatttt   3480
atgtatgtcc ccgtattaca cctccactgt cttggttcga caatttgcat aagttagaaa   3540
aattaatcat ccccggaaac aagaatatcg acgataattt cctcttacgg ctgtctcaga   3600
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tagtttgtat cgccctgaac tgccctaagc tgaagacgtt caacatcgga cgtcatcgcc   3720
gcggcaatct gattacatca gttagcttgg ttgccctggg taagtatacg caagttgaga   3780
ccgttggttt tgcaggctgc gatgtggacg acgcaggcat atgggagttc gcgcgtttaa   3840
acgggaaaaa cgtcgagcgc ctgtcactca acagttgccg gcttttaacc gactatagct   3900
tgccaatcct gtttgccctt aatagtttcc cgaaccttgc ggtgttggaa attcgaaacc   3960
tcgataaaat tacagatgtc cgccattttg tgaaatataa tctgtggaag aaatcactgg   4020
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accgggtcaa acgcataaat agcctggtcg ctttaaagga tatgaccgcg tgggtgaacg   4140
ctgacgatga aattgaaaac aacgtcgatt gagacgatga aattgaaaac aacgtcgatt   4200
gaggtaccat ggtttttgtg actttaccta taaatagtac acaacagacc accagtaatt   4260
ctacacactt cttaactgat aatattatta taattgtaac tttttagcag cactaaattt   4320
aatgaataca tagattttta actagcattt tactattctg tactttttac ttgaaattcc   4380
agaagggccg aagaaaccag aattccttca cagaaaacga attcactggc cgtcgtttta   4440
caacgtcgtg actgggaaaa ccctggcgtt acccaactta atcgccttgc agcacatccc   4500
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cgcagcctga atggcgaatg gcgcctgatg cggtattttc tccttacgca tctgtgcggt   4620
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cagccccgac acccgccaac acccgctgac gcgccctgac gggcttgtct gctcccggca   4740
tccgcttaca gacaagctgt gaccgtctcc gggagctgca tgtgtcagag gttttcaccg   4800
tcatcaccga aacgcgcgag acgaaagggc ctcgtgatac gcctattttt ataggttaat   4860
gtcatgataa taatggtttc ttagacgtca ggtggcactt ttcggggaaa tgtgcgcgga   4920
acccctattt gtttattttt ctaaatacat tcaaatatgt atccgctcat gagacaataa   4980
ccctgataaa tgcttcaata atattgaaaa aggaagagta tgagtattca acatttccgt   5040
gtcgccctta ttcccttttt tgcggcattt tgccttcctg tttttgctca cccagaaacg   5100
ctggtgaaag taaaagatgc tgaagatcag ttgggtgcac gagtgggtta catcgaactg   5160
gatctcaaca gcggtaagat ccttgagagt tttcgccccg aagaacgttt tccaatgatg   5220
agcactttta aagttctgct atgtggcgcg gtattatccc gtattgacgc cgggcaagag   5280
caactcggtc gccgcataca ctattctcag aatgacttgg ttgagtactc accagtcaca   5340
gaaaagcatc ttacggatgg catgacagta agagaattat gcagtgctgc cataaccatg   5400
agtgataaca ctgcggccaa cttacttctg acaacgatcg gaggaccgaa ggagctaacc   5460
gcttttttgc acaacatggg ggatcatgta actcgccttg atcgttggga accggagctg   5520
aatgaagcca taccaaacga cgagcgtgac accacgatgc ctgtagcaat ggcaacaacg   5580
ttgcgcaaac tattaactgg cgaactactt actctagctt cccggcaaca attaatagac   5640
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```
tggatggagg cggataaagt tgcaggacca cttctgcgct cggcccttcc ggctggctgg   5700

tttattgctg ataaatctgg agccggtgag cgtgggtctc gcggtatcat tgcagcactg   5760

gggccagatg gtaagccctc ccgtatcgta gttatctaca cgacggggag tcaggcaact   5820

atggatgaac gaaatagaca gatcgctgag ataggtgcct cactgattaa gcattggtaa   5880

ctgtcagacc aagtttactc atatatactt tagattgatt taaaacttca tttttaattt   5940

aaaaggatct aggtgaagat cctttttgat aatctcatga ccaaaatccc ttaacgtgag   6000

ttttcgttcc actgagcgtc agaccccgta gaaaagatca aaggatcttc ttgagatcct   6060

tttttctgc gcgtaatctg ctgcttgcaa acaaaaaaac caccgctacc agcggtggtt   6120

tgtttgccgg atcaagagct accaactctt tttccgaagg taactggctt cagcagagcg   6180

cagataccaa atactgtcct tctagtgtag ccgtagttag gccaccactt caagaactct   6240

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gataagtcgt gtcttaccgg gttggactca agacgatagt taccggataa ggcgcagcgg   6360

tcgggctgaa cggggggttc gtgcacacag cccagcttgg agcgaacgac ctacaccgaa   6420

ctgagatacc tacagcgtga gctatgagaa agcgccacgc ttcccgaagg gagaaaggcg   6480

gacaggtatc cggtaagcgg cagggtcgga acaggagagc gcacgaggga gcttccaggg   6540

ggaaacgcct ggtatcttta tagtcctgtc gggtttcgcc acctctgact tgagcgtcga   6600

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<210> SEQ ID NO 180
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA712

<400> SEQUENCE: 180

cttaattgaa agaaagaatt tccttcaact tcggtttcct ggttccgcta tttctcgctt     60

gtttcttcta gcattgcgga ttacgtattc taatgttcag                          100


<210> SEQ ID NO 181
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA746

<400> SEQUENCE: 181

gttttctgtg aaggaattct ggtttcttcg                                      30


<210> SEQ ID NO 182
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anaerostipes caccae KARI variant K9JB4P

<400> SEQUENCE: 182

Met Glu Glu Cys Lys Met Ala Lys Ile Tyr Tyr Gln Glu Asp Cys Asn
1               5                   10                  15

Leu Ser Leu Leu Asp Gly Lys Thr Ile Ala Val Ile Gly Tyr Gly Ser
            20                  25                  30

Gln Gly His Ala His Ala Leu Asn Ala Lys Glu Ser Gly Cys Asn Val
        35                  40                  45
```

```
Ile Ile Gly Leu Tyr Glu Gly Ala Glu Glu Trp Lys Arg Ala Glu Glu
    50                  55                  60

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

Ile Ile Met Ile Leu Ile Pro Asp Glu Lys Gln Ala Thr Met Tyr Lys
                85                  90                  95

Asn Asp Ile Glu Pro Asn Leu Glu Ala Gly Asn Met Leu Met Phe Ala
            100                 105                 110

His Gly Phe Asn Ile His Phe Gly Cys Ile Val Pro Pro Lys Asp Val
        115                 120                 125

Asp Val Thr Met Ile Ala Pro Lys Gly Pro Gly His Thr Val Arg Ser
    130                 135                 140

Glu Tyr Glu Glu Gly Lys Gly Val Pro Cys Leu Val Ala Val Glu Gln
145                 150                 155                 160

Asp Ala Thr Gly Lys Ala Leu Asp Met Ala Leu Ala Tyr Ala Leu Ala
                165                 170                 175

Ile Gly Gly Ala Arg Ala Gly Val Leu Glu Thr Thr Phe Arg Thr Glu
            180                 185                 190

Thr Glu Thr Asp Leu Phe Gly Glu Gln Ala Val Leu Cys Gly Gly Val
        195                 200                 205

Cys Ala Leu Met Gln Ala Gly Phe Glu Thr Leu Val Glu Ala Gly Tyr
    210                 215                 220

Asp Pro Arg Asn Ala Tyr Phe Glu Cys Ile His Glu Met Lys Leu Ile
225                 230                 235                 240

Val Asp Leu Ile Tyr Gln Ser Gly Phe Ser Gly Met Arg Tyr Ser Ile
                245                 250                 255

Ser Asn Thr Ala Glu Tyr Gly Asp Tyr Ile Thr Gly Pro Lys Ile Ile
            260                 265                 270

Thr Glu Asp Thr Lys Lys Ala Met Lys Lys Ile Leu Ser Asp Ile Gln
        275                 280                 285

Asp Gly Thr Phe Ala Lys Asp Phe Leu Val Asp Met Ser Asp Ala Gly
    290                 295                 300

Ser Gln Val His Phe Lys Ala Met Arg Lys Leu Ala Ser Glu His Pro
305                 310                 315                 320

Ala Glu Val Val Gly Glu Glu Ile Arg Ser Leu Tyr Ser Trp Ser Asp
                325                 330                 335

Glu Asp Lys Leu Ile Asn Asn
            340


<210> SEQ ID NO 183
<211> LENGTH: 571
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Streptococcus mutans DHAD variant L2V4

<400> SEQUENCE: 183

Met Thr Asp Lys Lys Thr Leu Lys Asp Leu Arg Asn Arg Ser Ser Val
1               5                   10                  15

Tyr Asp Ser Met Val Lys Ser Pro Asn Arg Ala Met Leu Arg Ala Thr
            20                  25                  30

Gly Met Gln Asp Glu Asp Phe Glu Lys Pro Ile Val Gly Val Ile Ser
        35                  40                  45

Thr Trp Ala Glu Asn Thr Pro Cys Asn Ile His Leu His Asp Phe Gly
    50                  55                  60
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Lys Leu Ala Lys Val Gly Val Lys Glu Ala Gly Ala Trp Pro Val Gln
65                  70                  75                  80

Phe Gly Thr Ile Thr Val Ser Asp Gly Ile Ala Met Gly Thr Gln Gly
                85                  90                  95

Met Arg Phe Ser Leu Thr Ser Arg Asp Ile Ile Ala Asp Ser Ile Glu
            100                 105                 110

Ala Ala Met Gly Gly His Asn Ala Asp Ala Phe Val Ala Ile Gly Gly
        115                 120                 125

Cys Asp Lys Asn Met Pro Gly Ser Val Ile Ala Met Ala Asn Met Asp
    130                 135                 140

Ile Pro Ala Ile Phe Ala Tyr Gly Gly Thr Ile Ala Pro Gly Asn Leu
145                 150                 155                 160

Asp Gly Lys Asp Ile Asp Leu Val Ser Val Phe Glu Gly Val Gly His
                165                 170                 175

Trp Asn His Gly Asp Met Thr Lys Glu Glu Val Lys Ala Leu Glu Cys
            180                 185                 190

Asn Ala Cys Pro Gly Pro Gly Gly Cys Gly Gly Met Tyr Thr Ala Asn
        195                 200                 205

Thr Met Ala Thr Ala Ile Glu Val Leu Gly Leu Ser Leu Pro Gly Ser
    210                 215                 220

Ser Ser His Pro Ala Glu Ser Ala Glu Lys Lys Ala Asp Ile Glu Glu
225                 230                 235                 240

Ala Gly Arg Ala Val Val Lys Met Leu Glu Met Gly Leu Lys Pro Ser
                245                 250                 255

Asp Ile Leu Thr Arg Glu Ala Phe Glu Asp Ala Ile Thr Val Thr Met
            260                 265                 270

Ala Leu Gly Gly Ser Thr Asn Ser Thr Leu His Leu Leu Ala Ile Ala
        275                 280                 285

His Ala Ala Asn Val Glu Leu Thr Leu Asp Asp Phe Asn Thr Phe Gln
    290                 295                 300

Glu Lys Val Pro His Leu Ala Asp Leu Lys Pro Ser Gly Gln Tyr Val
305                 310                 315                 320

Phe Gln Asp Leu Tyr Lys Val Gly Gly Val Pro Ala Val Met Lys Tyr
                325                 330                 335

Leu Leu Lys Asn Gly Phe Leu His Gly Asp Arg Ile Thr Cys Thr Gly
            340                 345                 350

Lys Thr Val Ala Glu Asn Leu Lys Ala Phe Asp Asp Leu Thr Pro Gly
        355                 360                 365

Gln Lys Val Ile Met Pro Leu Glu Asn Pro Lys Arg Glu Asp Gly Pro
    370                 375                 380

Val Ile Ile Leu His Gly Asn Leu Ala Pro Asp Gly Ala Val Ala Lys
385                 390                 395                 400

Val Ser Gly Val Lys Val Arg Arg His Val Gly Pro Ala Lys Val Phe
                405                 410                 415

Asn Ser Glu Glu Glu Ala Ile Glu Ala Val Leu Asn Asp Asp Ile Val
            420                 425                 430

Asp Gly Asp Val Val Val Val Arg Phe Val Gly Pro Lys Gly Gly Pro
        435                 440                 445

Gly Met Pro Glu Met Leu Ser Leu Ser Ser Met Ile Val Gly Lys Gly
    450                 455                 460

Gln Gly Glu Lys Val Ala Leu Leu Thr Asp Gly Arg Phe Ser Gly Gly
465                 470                 475                 480
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Thr Tyr Gly Leu Val Val Gly His Ile Ala Pro Glu Ala Gln Asp Gly
                485                 490                 495

Gly Pro Ile Ala Tyr Leu Gln Thr Gly Asp Ile Val Thr Ile Asp Gln
            500                 505                 510

Asp Thr Lys Glu Leu His Phe Asp Ile Ser Asp Glu Glu Leu Lys His
        515                 520                 525

Arg Gln Glu Thr Ile Glu Leu Pro Pro Leu Tyr Ser Arg Gly Ile Leu
    530                 535                 540

Gly Lys Tyr Ala His Ile Val Ser Ser Ala Ser Arg Gly Ala Val Thr
545                 550                 555                 560

Asp Phe Trp Lys Pro Glu Glu Thr Gly Lys Lys
                565                 570


&lt;210&gt; SEQ ID NO 184
&lt;211&gt; LENGTH: 7523
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Artificial Sequence
&lt;220&gt; FEATURE:
&lt;223&gt; OTHER INFORMATION: pLA34

&lt;400&gt; SEQUENCE: 184

ccagcttttg ttccctttag tgagggttaa ttgcgcgctt ggcgtaatca tggtcatagc     60

tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatagga gccggaagca    120

taaagtgtaa agcctggggt gcctaatgag tgaggtaact cacattaatt gcgttgcgct    180

cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga atcggccaac    240

gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc actgactcgc    300

tgcgctcggt cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg gtaatacggt    360

tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg    420

ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc ccccctgacg    480

agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga ctataaagat    540

accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc ctgccgctta    600

ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat agctcacgct    660

gtaggtatct cagttcggtg taggtcgttc gctccaagct gggctgtgtg cacgaacccc    720

ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc aacccggtaa    780

gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg    840

taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact agaaggacag    900

tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt    960

gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag cagcagatta   1020

cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg tctgacgctc   1080

agtggaacga aaactcacgt taagggattt tggtcatgag attatcaaaa aggatcttca   1140

cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata tatgagtaaa   1200

cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg atctgtctat   1260

ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata cgggagggct   1320

taccatctgg ccccagtgct gcaatgatac cgcgagaccc acgctcaccg gctccagatt   1380

tatcagcaat aaaccagcca gccggaaggg ccgagcgcag aagtggtcct gcaactttat   1440

ccgcctccat ccagtctatt aattgttgcc gggaagctag agtaagtagt tcgccagtta   1500

atagtttgcg caacgttgtt gccattgcta caggcatcgt ggtgtcacgc tcgtcgtttg   1560
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gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga tcccccatgt   1620
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cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc atgccatccg   1740
taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa tagtgtatgc   1800
ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca catagcagaa   1860
ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca aggatcttac   1920
cgctgttgag atccagttcg atgtaaccca ctcgtgcacc caactgatct tcagcatctt   1980
ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg   2040
gaataagggc gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa   2100
gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata   2160
aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgaacga agcatctgtg   2220
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atctgagctg catttttaca gaacagaaat gcaacgcgag agcgctattt taccaacaaa   2460
gaatctatac ttcttttttg ttctacaaaa atgcatcccg agagcgctat ttttctaaca   2520
aagcatctta gattactttt tttctccttt gtgcgctcta taatgcagtc tcttgataac   2580
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cgattcactc tatgaatagt tcttactaca atttttttgt ctaaagagta atactagaga   2940
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ggtaggttat atagggatat agcacagaga tatatagcaa agagatactt ttgagcaatg   3060
tttgtggaag cggtattcgc aatattttag tagctcgtta cagtccggtg cgtttttggt   3120
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ctttctagag aataggaact tcggaatagg aacttcaaag cgtttccgaa aacgagcgct   3240
tccgaaaatg caacgcgagc tgcgcacata cagctcactg ttcacgtcgc acctatatct   3300
gcgtgttgcc tgtatatata tatacatgag aagaacggca tagtgcgtgt ttatgcttaa   3360
atgcgtactt atatgcgtct atttatgtag gatgaaaggt agtctagtac ctcctgtgat   3420
attatcccat tccatgcggg gtatcgtatg cttccttcag cactaccctt tagctgttct   3480
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attggatcat ctaagaaacc attattatca tgacattaac ctataaaaat aggcgtatca   3600
cgaggccctt tcgtctcgcg cgtttcggtg atgacggtga aaacctctga cacatgcagc   3660
tcccggagac ggtcacagct tgtctgtaag cggatgccgg gagcagacaa gcccgtcagg   3720
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ttgtactgag agtgcaccat aaattcccgt tttaagagct tggtgagcgc taggagtcac   3840
tgccaggtat cgtttgaaca cggcattagt cagggaagtc ataacacagt cctttcccgc   3900
```

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aattttcttt ttctattact cttggcctcc tctagtacac tctatatttt tttatgcctc   3960
ggtaatgatt ttcatttttt tttttcccct agcggatgac tctttttttt tcttagcgat   4020
tggcattatc acataatgaa ttatacatta tataaagtaa tgtgatttct tcgaagaata   4080
tactaaaaaa tgagcaggca agataaacga aggcaaagat gacagagcag aaagccctag   4140
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ggttgagtgt tgttccagtt tggaacaaga gtccactatt aaagaacgtg gactccaacg   5220
tcaaagggcg aaaaaccgtc tatcagggcg atggcccact acgtgaacca tcaccctaat   5280
caagtttttt ggggtcgagg tgccgtaaag cactaaatcg gaaccctaaa gggagccccc   5340
gatttagagc ttgacgggga aagccggcga acgtggcgag aaaggaaggg aagaaagcga   5400
aaggagcggg cgctagggcg ctggcaagtg tagcggtcac gctgcgcgta accaccacac   5460
ccgccgcgct taatgcgccg ctacagggcg cgtcgcgcca ttcgccattc aggctgcgca   5520
actgttggga agggcgatcg gtgcgggcct cttcgctatt acgccagctg gcgaaagggg   5580
gatgtgctgc aaggcgatta agttgggtaa cgccagggtt ttcccagtca cgacgttgta   5640
aaacgacggc cagtgagcgc gcgtaatacg actcactata gggcgaattg ggtaccgggc   5700
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tccgtgcgtc ctcgtcttca ccggtcgcgt tcctgaaacg cagatgtgcc tcgcgccgca   5820
ctgctccgaa caataaagat tctacaatac tagcttttat ggttatgaag aggaaaaatt   5880
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ataatgcgat tagtttttta gccttatttc tggggtaatt aatcagcgaa gcgatgattt   6000
ttgatctatt aacagatata taaatggaaa agctgcataa ccactttaac taatactttc   6060
aacattttca gtttgtatta cttcttattc aaatgtcata aaagtatcaa caaaaaattg   6120
ttaatatacc tctatacttt aacgtcaagg agaaaaaatgt ccaatttact gcccgtacac   6180
caaaatttgc ctgcattacc ggtcgatgca acgagtgatg aggttcgcaa gaacctgatg   6240
gacatgttca gggatcgcca ggcgttttct gagcatacct ggaaaatgct tctgtccgtt   6300
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tgccggtcgt gggcggcatg gtgcaagttg aataaccgga aatggtttcc cgcagaacct   6360
gaagatgttc gcgattatct tctatatctt caggcgcgcg gtctggcagt aaaaactatc   6420
cagcaacatt tgggccagct aaacatgctt catcgtcggt ccgggctgcc acgaccaagt   6480
gacagcaatg ctgtttcact ggttatgcgg cggatccgaa aagaaaacgt tgatgccggt   6540
gaacgtgcaa aacaggctct agcgttcgaa cgcactgatt tcgaccaggt tcgttcactc   6600
atggaaaata gcgatcgctg ccaggatata cgtaatctgg catttctggg gattgcttat   6660
aacaccctgt tacgtatagc cgaaattgcc aggatcaggg ttaaagatat ctcacgtact   6720
gacggtggga gaatgttaat ccatattggc agaacgaaaa cgctggttag caccgcaggt   6780
gtagagaagg cacttagcct gggggtaact aaactggtcg agcgatggat ttccgtctct   6840
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gcgccatctg ccaccagcca gctatcaact cgcgccctgg aagggatttt tgaagcaact   6960
catcgattga tttacggcgc taaggatgac tctggtcaga gatacctggc ctggtctgga   7020
cacagtgccc gtgtcggagc cgcgcgagat atggcccgcg ctggagtttc aataccggag   7080
atcatgcaag ctggtggctg gaccaatgta aatattgtca tgaactatat ccgtaacctg   7140
gatagtgaaa caggggcaat ggtgcgcctg ctggaagatg gcgattagga gtaagcgaat   7200
ttcttatgat ttatgatttt tattattaaa taagttataa aaaaaataag tgtatacaaa   7260
ttttaaagtg actcttaggt tttaaaacga aaattcttat tcttgagtaa ctctttcctg   7320
taggtcaggt tgctttctca ggtatagcat gaggtcgctc ttattgacca cacctctacc   7380
ggcatgccga gcaaatgcct gcaaatcgct ccccatttca cccaattgta gatatgctaa   7440
ctccagcaat gagttgatga atctcggtgt gtattttatg tcctcagagg acaacacctg   7500
tggtccgcca ccgcggtgga gct                                           7523
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<210> SEQ ID NO 185
<211> LENGTH: 96
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA722

<400> SEQUENCE: 185

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tgccaattat ttacctaaac atctataacc ttcaaaagta aaaaaataca caaacgttga     60
atcatcacct tggctaactc gttgtatcat cactgg                               96
```

<210> SEQ ID NO 186
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA733

<400> SEQUENCE: 186

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cataatcaat ctcaaagaga acaacacaat acaataacaa gaagaacaaa gcattgcgga     60
ttacgtattc taatgttcag                                                 80
```

<210> SEQ ID NO 187
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA453

<400> SEQUENCE: 187

caccgaagaa gaatgcaaaa atttcagctc 30

<210> SEQ ID NO 188
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA694

<400> SEQUENCE: 188

gctgaagttg ttagaactgt tgttg 25

<210> SEQ ID NO 189
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA695

<400> SEQUENCE: 189

tgttagctgg agtagacttg g 21

<210> SEQ ID NO 190
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP594

<400> SEQUENCE: 190

agctgtctcg tgttgtgggt tt 22

<210> SEQ ID NO 191
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP595

<400> SEQUENCE: 191

cttaataata gaacaatatc atcctttacg ggcatcttat agtgtcgtt 49

<210> SEQ ID NO 192
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP596

<400> SEQUENCE: 192

gcgccaacga cactataaga tgcccgtaaa ggatgatatt gttctatta 49

<210> SEQ ID NO 193
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP597

<400> SEQUENCE: 193

tatggaccct gaaaccacag ccacattgca acgacgacaa tgccaaacc 49

<210> SEQ ID NO 194
<211> LENGTH: 49

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP598

<400> SEQUENCE: 194

tccttggttt ggcattgtcg tcgttgcaat gtggctgtgg tttcagggt 49

<210> SEQ ID NO 195
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP599

<400> SEQUENCE: 195

atcctctcgc ggagtccctg ttcagtaaag gccatgaagc tttttcttt 49

<210> SEQ ID NO 196
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP600

<400> SEQUENCE: 196

attggaaaga aaaagcttca tggcctttac tgaacaggga ctccgcgag 49

<210> SEQ ID NO 197
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP601

<400> SEQUENCE: 197

tcataccaca atcttagacc at 22

<210> SEQ ID NO 198
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP602

<400> SEQUENCE: 198

tgttcaaacc cctaaccaac c 21

<210> SEQ ID NO 199
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oBP603

<400> SEQUENCE: 199

tgttcccaca atctattacc ta 22

<210> SEQ ID NO 200
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA811

<400> SEQUENCE: 200

aacgaagcat ctgtgcttca ttttgtagaa c 31

<210> SEQ ID NO 201
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA817

<400> SEQUENCE: 201

cgatccactt gtatatttgg atgaattttt gaggaattct gaaccagtcc taaaacgag 59

<210> SEQ ID NO 202
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA812

<400> SEQUENCE: 202

aacaaagata tgctattgaa gtgcaagatg g 31

<210> SEQ ID NO 203
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LA818

<400> SEQUENCE: 203

ctcaaaaatt catccaaata tacaagtgga tcg 33

<210> SEQ ID NO 204
<211> LENGTH: 90
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<223> OTHER INFORMATION: 366

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```

What is claimed:

1. A method for production of isobutanol in a fermentation process comprising:

providing a fermentation mix comprising a recombinant yeast production microorganism which comprises an engineered isobutanol biosynthetic pathway, a heterologous polynucleotide encoding a polypeptide having acetolactate synthase activity, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 9 and confers resistant to sulfonylureas, and a heterologous polynucleotide encoding a polypeptide having 3-phosphoshikimate 1-carboxylvinyltransferase activity; and

contacting the fermentation mix with at least one sulfonylurea which preferentially inhibits at least one contaminant yeast microorganism, wherein a fermentation product of the contaminant yeast microorganism is ethanol;

wherein production competitiveness of the recombinant yeast production microorganism is associated with a higher isobutanol-to-ethanol ratio as compared to a fermentation process without addition of one or more inhibitors, antibiotics, or combinations thereof.

2. The method of claim **1**, wherein the specific growth rate of the at least one contaminant yeast microorganism is reduced more than the specific growth rate of the recombinant yeast production microorganism.

3. The method of claim **1**, wherein production of the fermentation product of the at least one contaminant yeast microorganism is reduced more than the isobutanol production of the recombinant yeast production microorganism.

4. The method of claim **1**, wherein the contaminant yeast microorganism is *Saccharomyces cerevisiae*.

5. The method of claim **1**, wherein the sulfonylurea is an inhibitor of an ethanol biosynthesis pathway.

6. The method of claim **1**, wherein the sulfonylurea is an inhibitor of an amino acid biosynthesis pathway.

7. The method of claim **1**, wherein the sulfonylurea is selected from a group consisting of: nicosulfuron methyl, metsulfuron methyl, chlorimuron ethyl, sulfometuron methyl, chlorsulfuron, thifensulfuron methyl, and mixtures thereof.

8. The method of claim **1**, wherein the recombinant yeast production microorganism is selected from *Schizosaccharomyces, Issatchenkia, Kluyveromyces, Yarrowia, Pichia, Candida, Hansenula, Aspergillus, Pachysolen, Rhodotorula, Zygosaccharomyces, Galactomyces, Torulaspora, Debayomyces, Williopsis, Dekkera, Kloeckera, Metschnikowia*, and *Saccharomyces*.

9. The method of claim **1**, wherein the isobutanol biosynthetic pathway comprises the following substrate to product conversions:

a) pyruvate to acetolactate;

b) acetolactate to 2,3-dihydroxyisovalerate;

c) 2,3-dihydroxyisovalerate to α-ketoisovalerate;

d) α-ketoisovalerate to isobutyraldehyde; and

e) isobutyraldehyde to isobutanol.

10. The method of claim **1**, wherein the recombinant yeast production microorganism further comprises one or more of the following modifications:

a deletion in one or more endogenous polynucleotides encoding a polypeptide having pyruvate decarboxylase activity;

a deletion, mutation, or substitution in an endogenous polynucleotide encoding a polypeptide having acetolactate reductase activity;

a deletion, mutation, or substitution in an endogenous polynucleotide encoding a polypeptide having aldehyde dehydrogenase activity;

a deletion in an endogenous polynucleotide encoding a polypeptide having hexokinase activity;

a deletion in an endogenous polynucleotide encoding a polypeptide having glycerol-3-phosphate dehydrogenase activity; or

a deletion in an endogenous gene encoding a polypeptide affecting Fe-S cluster biosynthesis, wherein the polypeptide is FRA2.

* * * * *