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Reed et al.

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(54) **MICROORGANISMS THAT CO-CONSUME GLUCOSE WITH NON-GLUCOSE CARBOHYDRATES AND METHODS OF USE**

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C12N 1/14	(2006.01)
C12P 7/06	(2006.01)
C12N 9/88	(2006.01)
C12N 9/04	(2006.01)

(52) **U.S. Cl.**

CPC ***C12P 7/065*** (2013.01); ***C12N 1/14*** (2013.01); ***C12N 1/20*** (2013.01); ***C12N 9/0006*** (2013.01); ***C12N 9/88*** (2013.01); ***C12P 7/06*** (2013.01); *C12Y 101/01001* (2013.01); *C12Y 401/01001* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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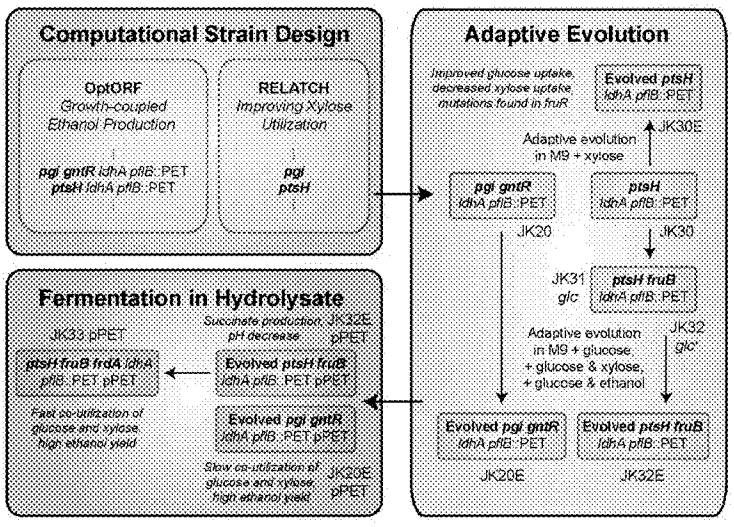
(57) **ABSTRACT**

Microorganisms that co-consume glucose with non-glucose carbohydrates, such as xylose, and methods of using same. The microorganisms comprise modifications that reduce or ablate the activity of a phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) protein or modifications that reduce or ablate the activity of a phosphoglucose isomerase and a GntR. The PTS protein may be selected from an enzyme I (EI), an HPr, an FPr, and an enzyme II^{Glc} (EII^{Glc}). Additional modifications include reduction or ablation of the activity of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase and inclusion of recombinant pyruvate decarboxylase and alcohol dehydrogenase genes. The microorganisms are particularly suited to co-consuming glucose and xylose in media containing these substrates and producing ethanol therefrom.

20 Claims, 20 Drawing Sheets

Specification includes a Sequence Listing.



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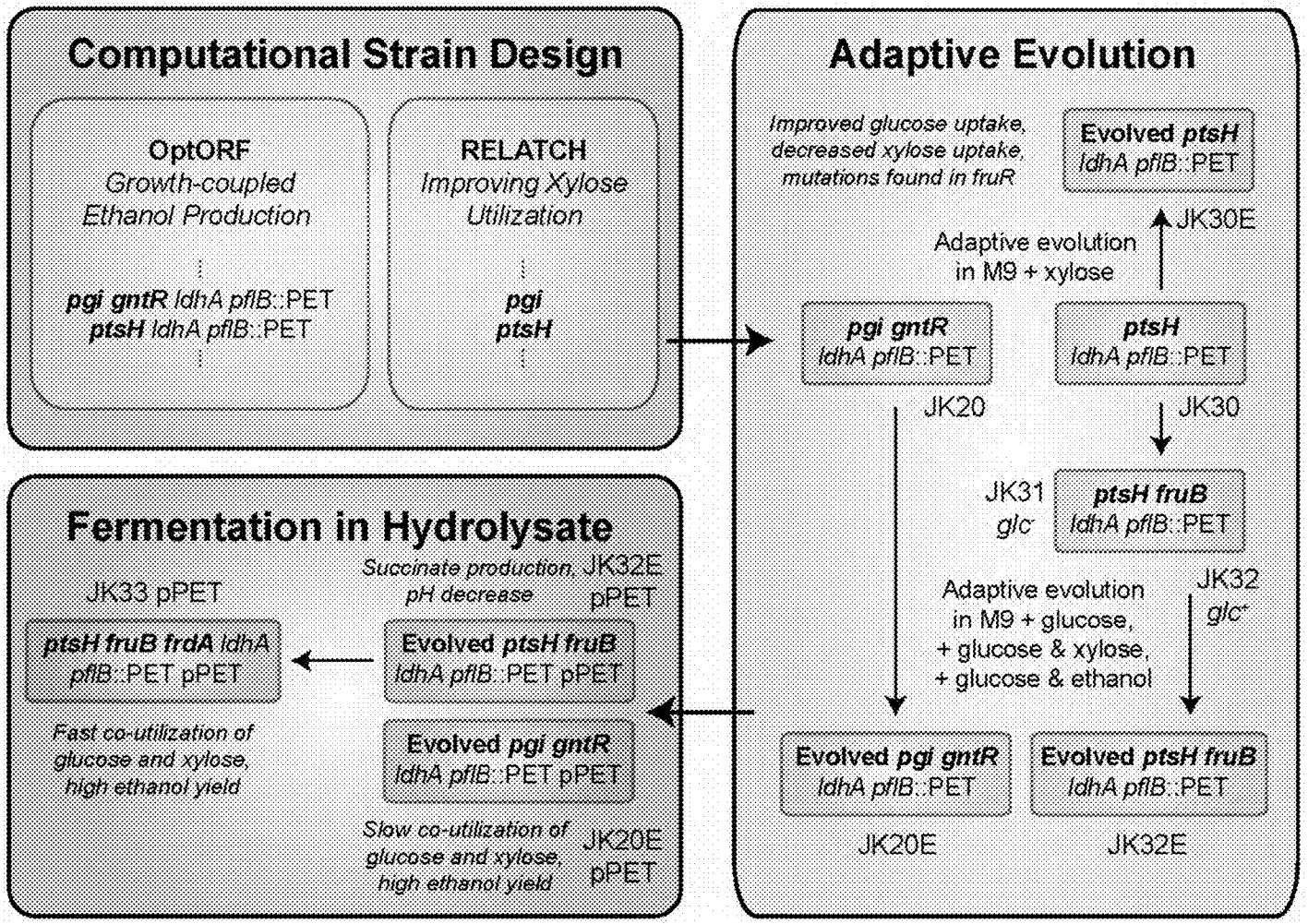
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ptsH ldhA pflB::PET
RELATCH
Improving Xylose
Utilization
pgi
ptsH
Adaptive Evolution
Improved glucose uptake, decreased xylose uptake, mutations found in fruR
Evolved ptsH ldhA pflB::PET
JK30E
Adaptive evolution in M9 + xylose
pgi gntR ldhA pflB::PET
ptsH ldhA pflB::PET
JK20
JK30
JK31 glc-
ptsH fruB ldhA pflB::PET
Adaptive evolution in M9 + glucose, + glucose & xylose, + glucose & ethanol
JK32 glc+
Evolved pgi gntR ldhA pflB::PET
Evolved ptsH fruB ldhA pflB::PET
JK20E
JK32E
Fermentation in Hydrolysate
Succinate production, pH decrease
JK32E pPET
JK33 pPET
ptsH fruB frdA ldhA pflB::PET pPET
Evolved ptsH fruB ldhA pflB::PET pPET
Fast co-utilization of glucose and xylose, high ethanol yield
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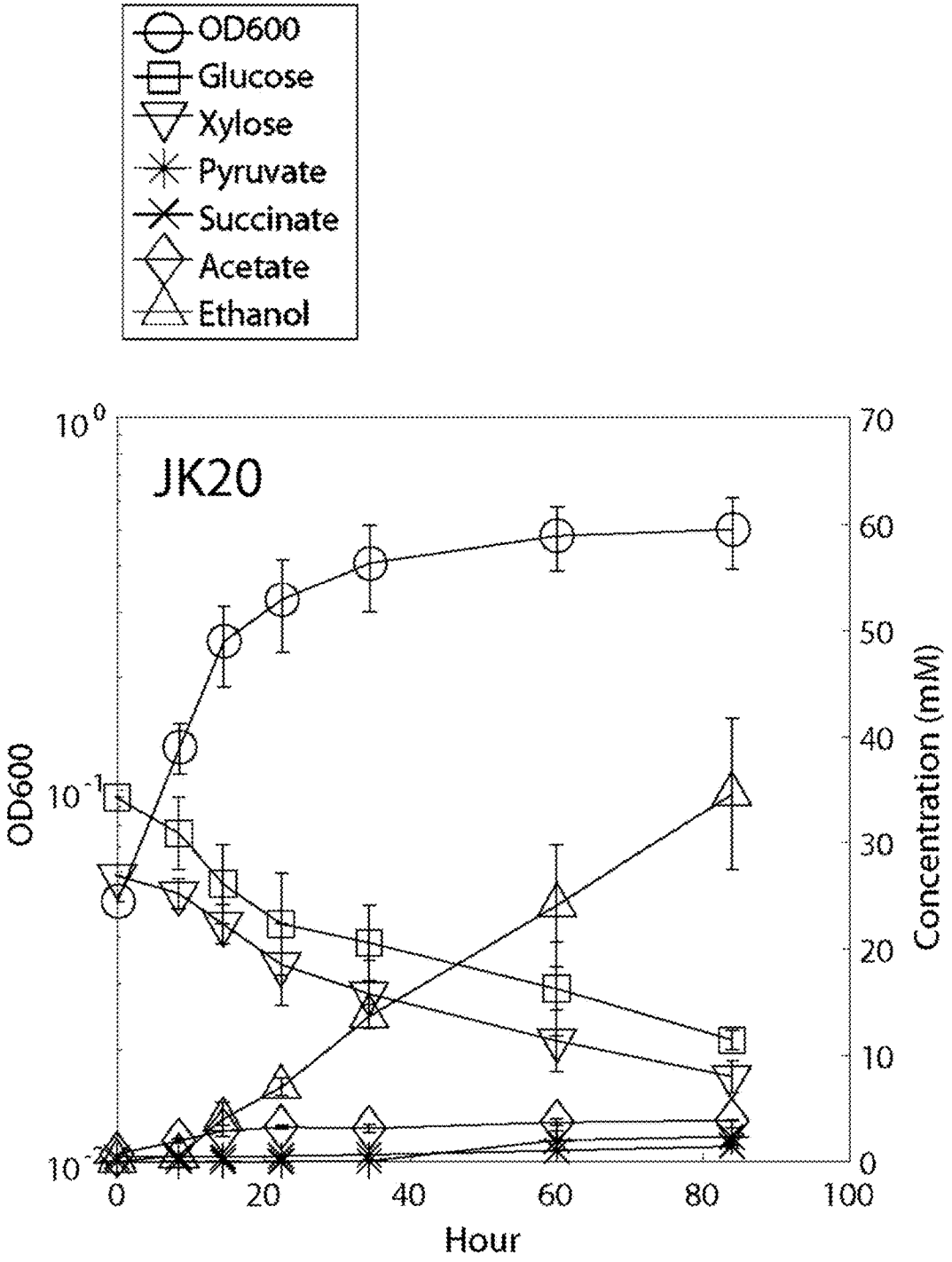
Slow co-utilization of glucose and xylose, high ethanol yield
JK20E pPET

FIG. 1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Xylose
Pyruvate
Succinate
Acetate
Ethanol
JK20
OD600
Concentration (mM)
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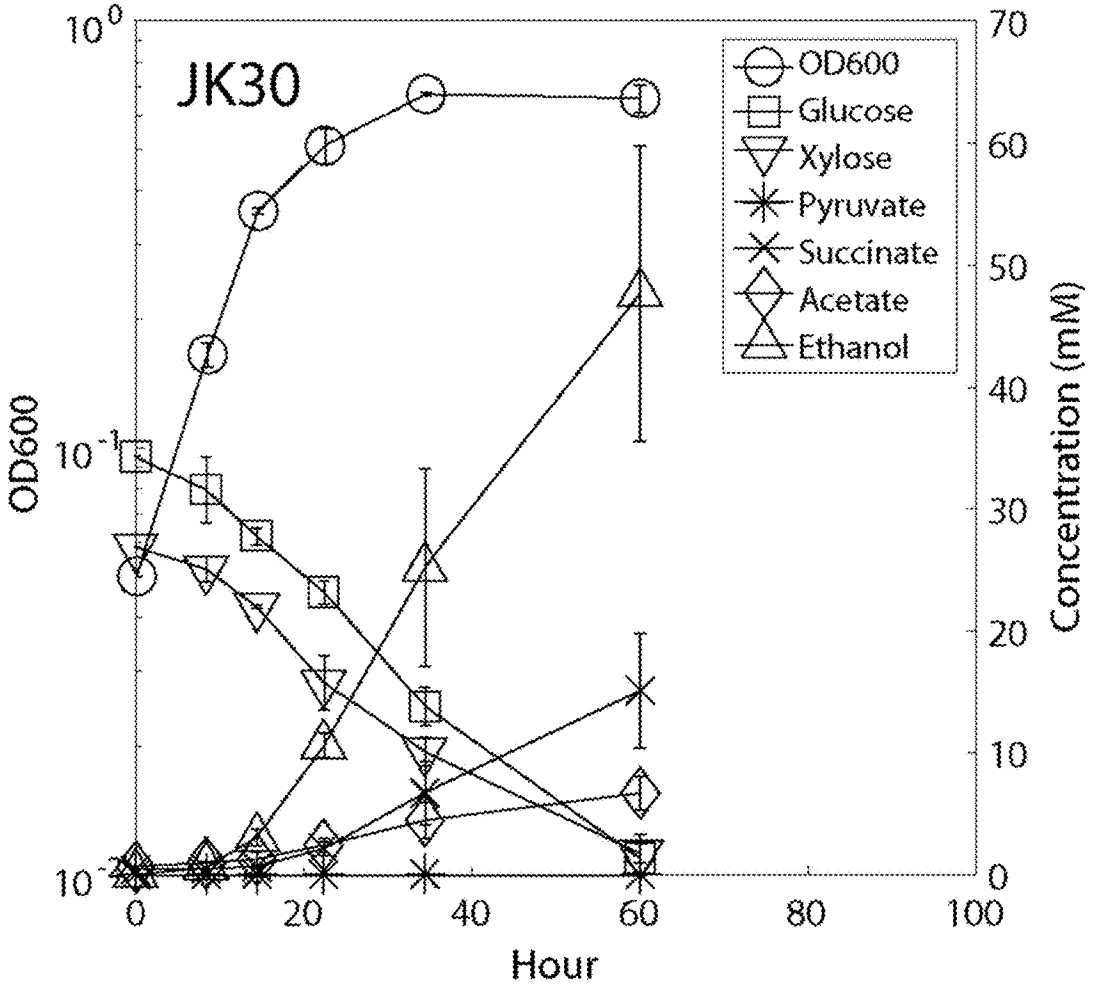
FIG. 2A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Hour
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FIG. 2B

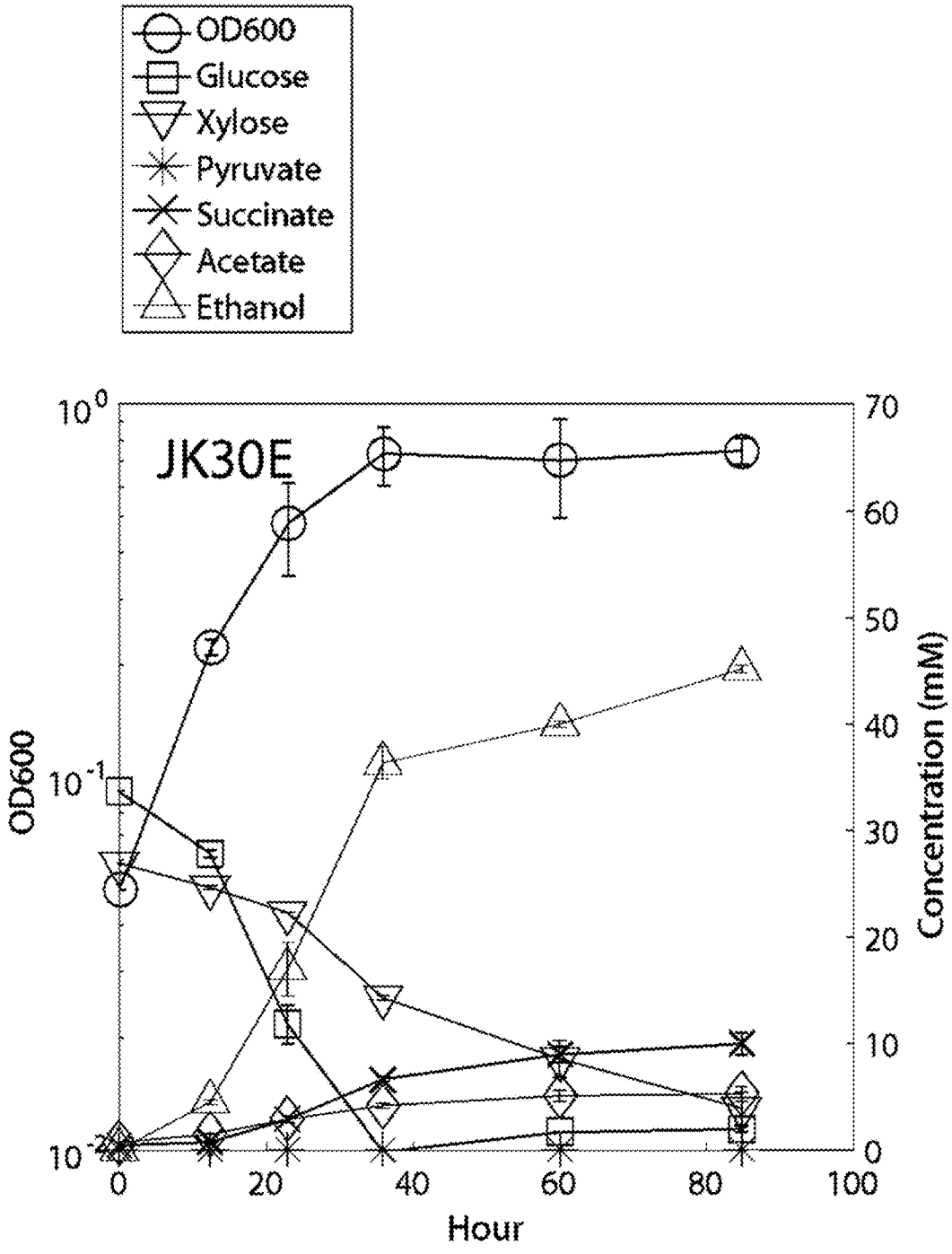
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FIG. 2C

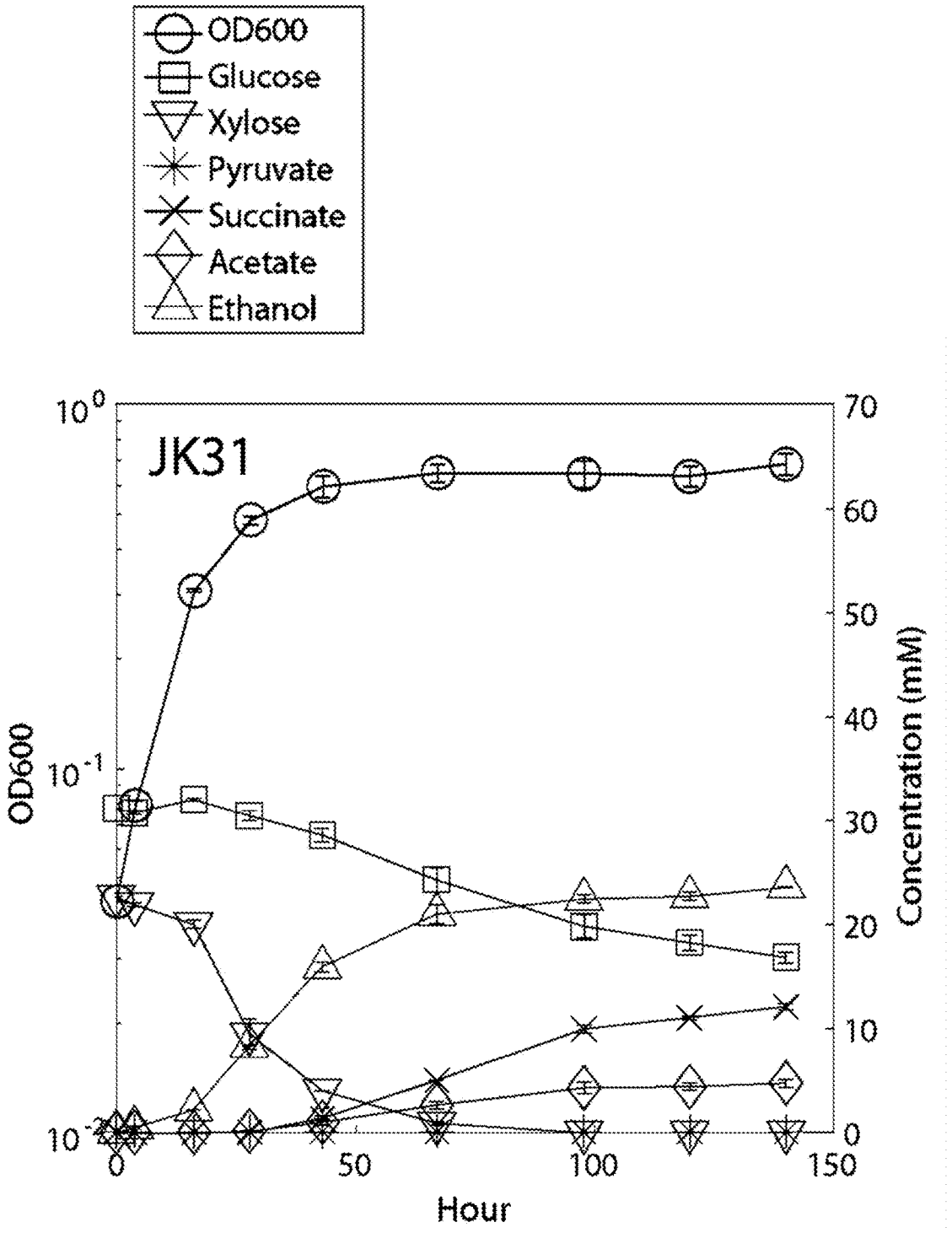
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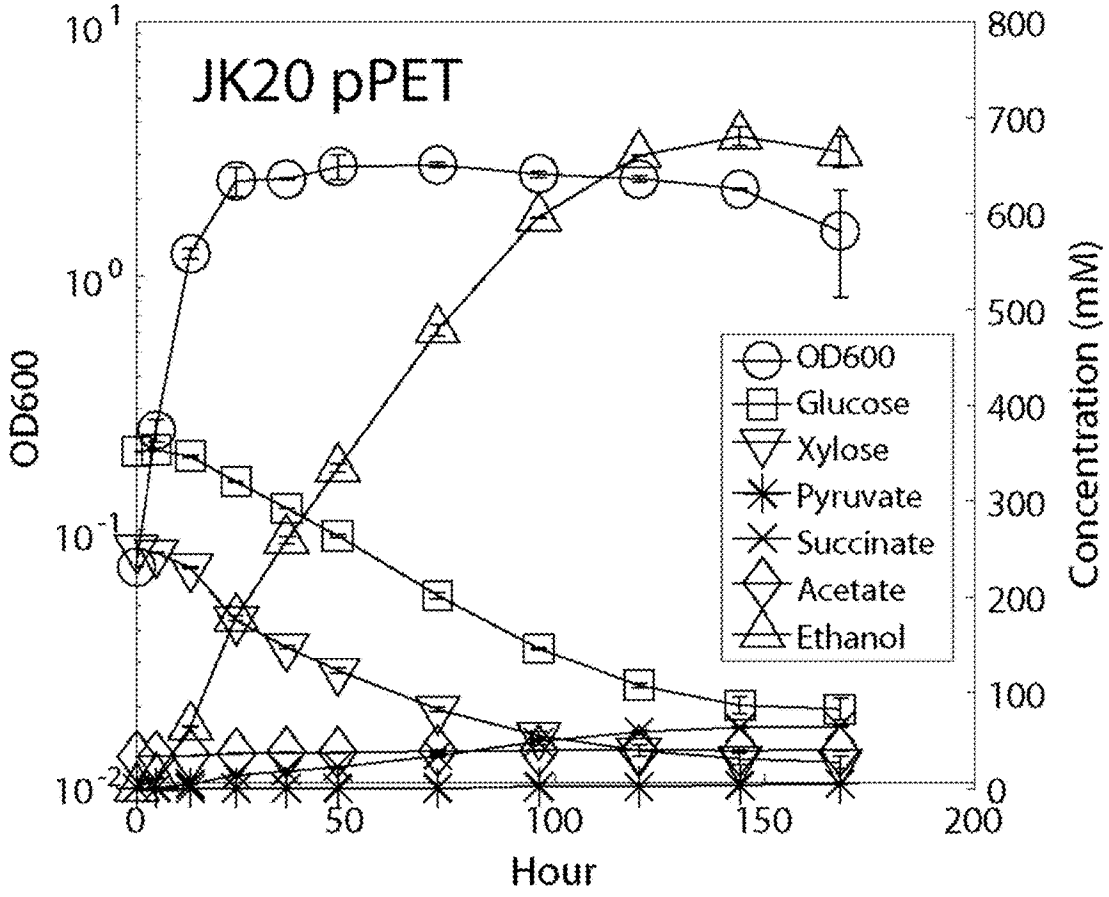
FIG. 2D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Ethanol
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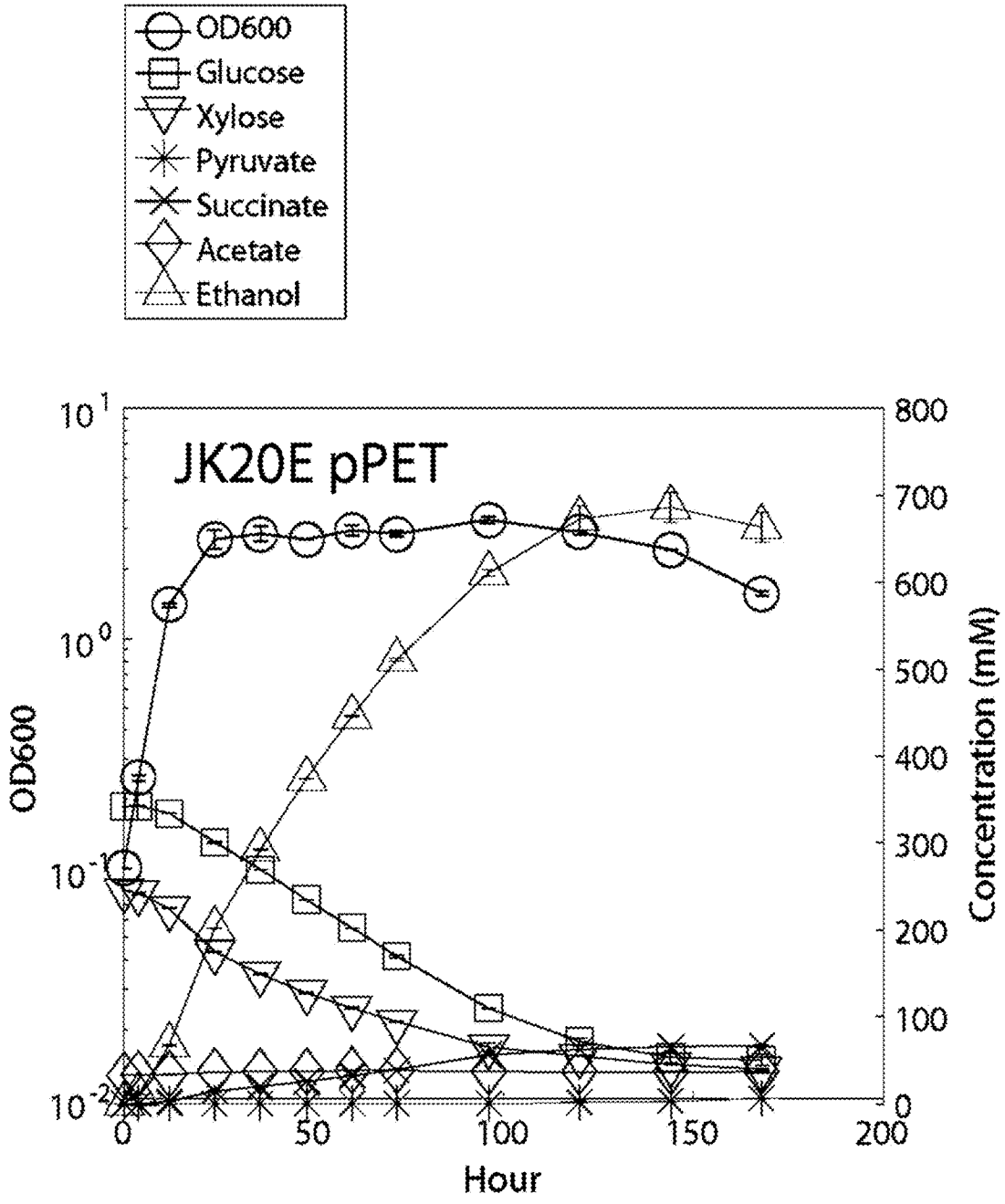
150
200

FIG. 3A


OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
JK20E pPET
OD600
Concentration (mM)
Hour
10^{1}
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10^{-1}
10^{-2}
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200

FIG. 3B

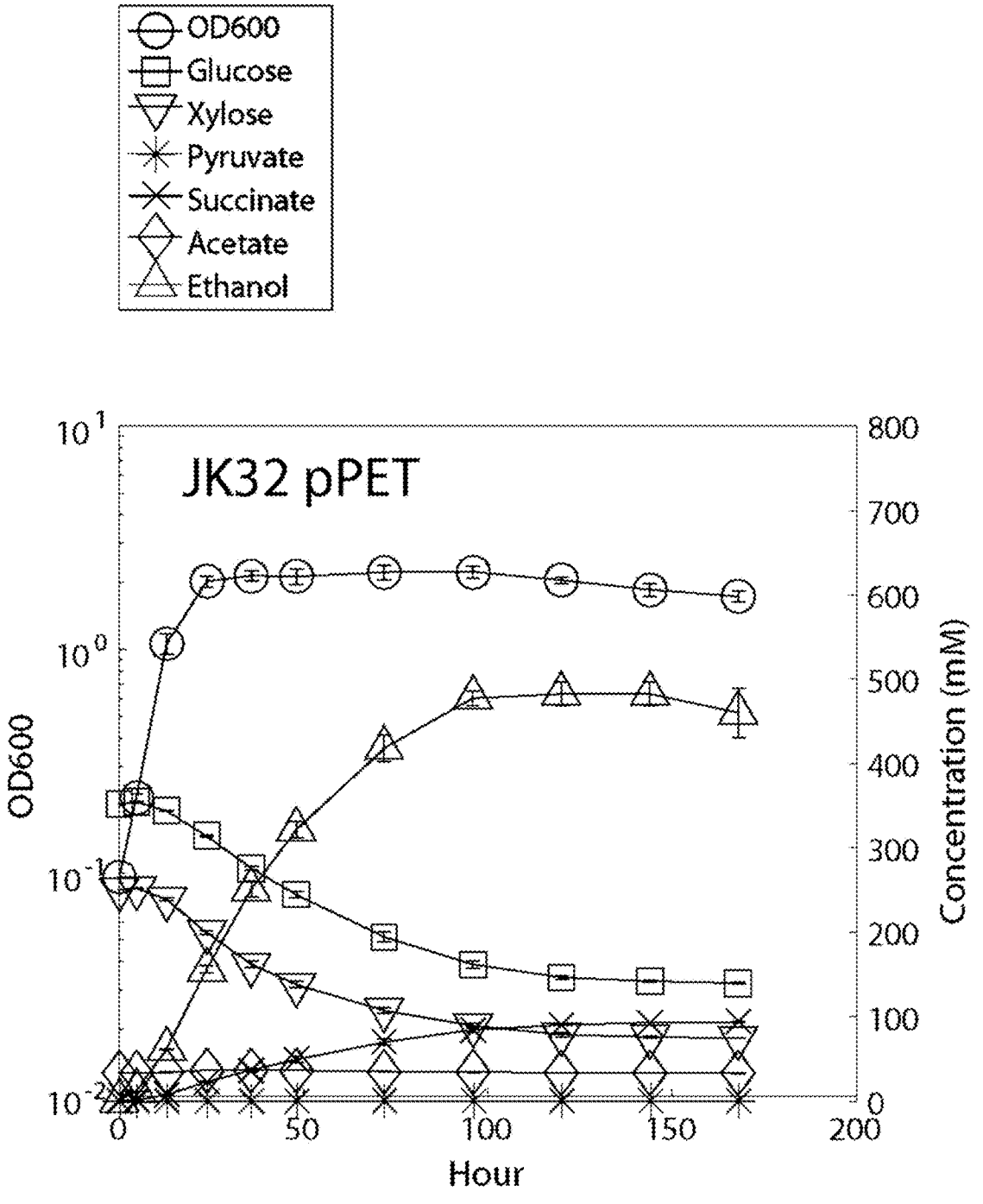
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
JK32 pPET
OD600
Concentration (mM)
Hour
10^1
10^0
10^{-1}
10^{-2}
800
700
600
500
400
300
200
100
0
0
50
100
150
200

FIG. 3C

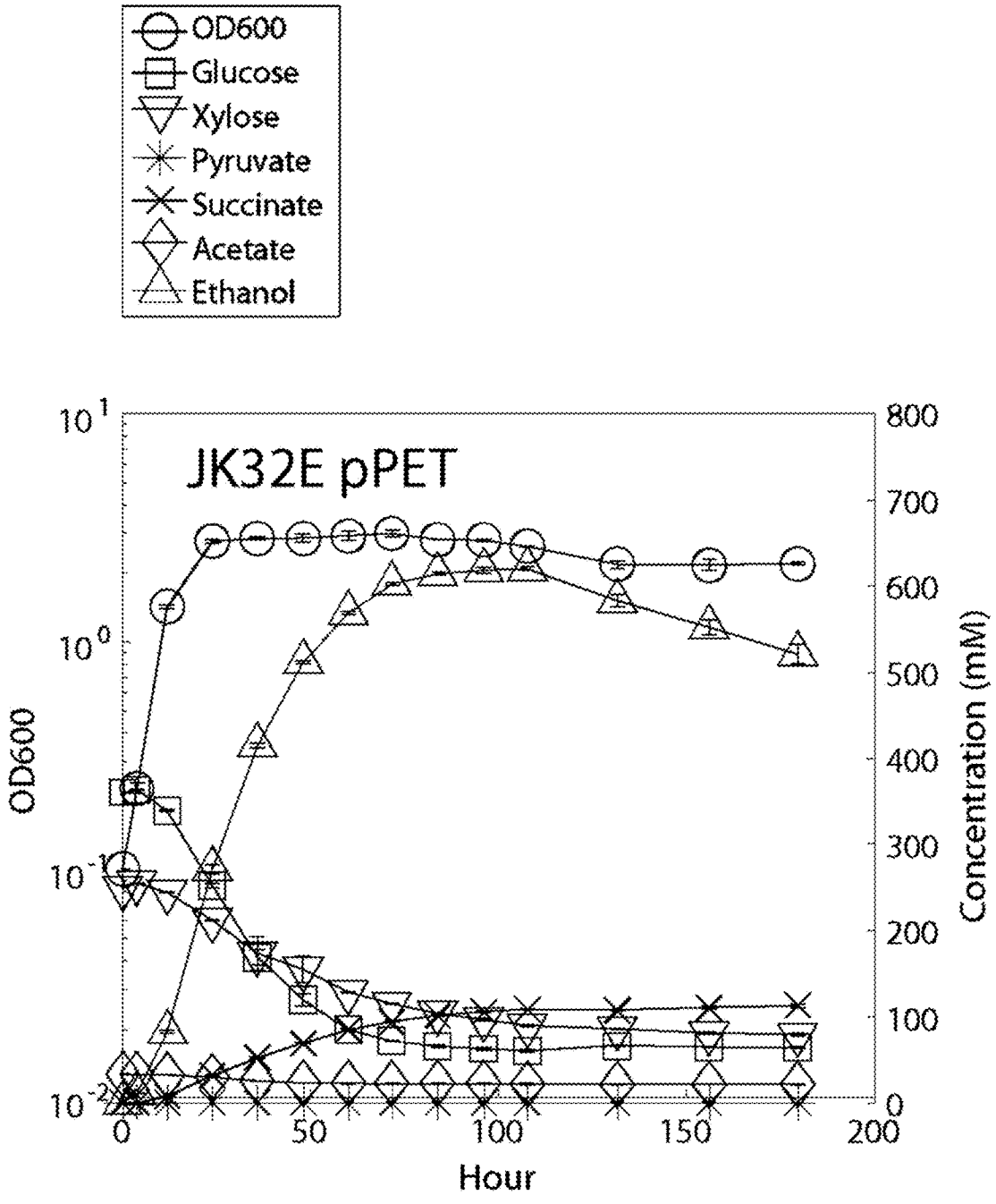
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
JK32E pPET
OD600
Concentration (mM)
Hour
10^1
10^0
10^-1
10^-2
800
700
600
500
400
300
200
100
0
0
50
100
150
200

FIG. 3D

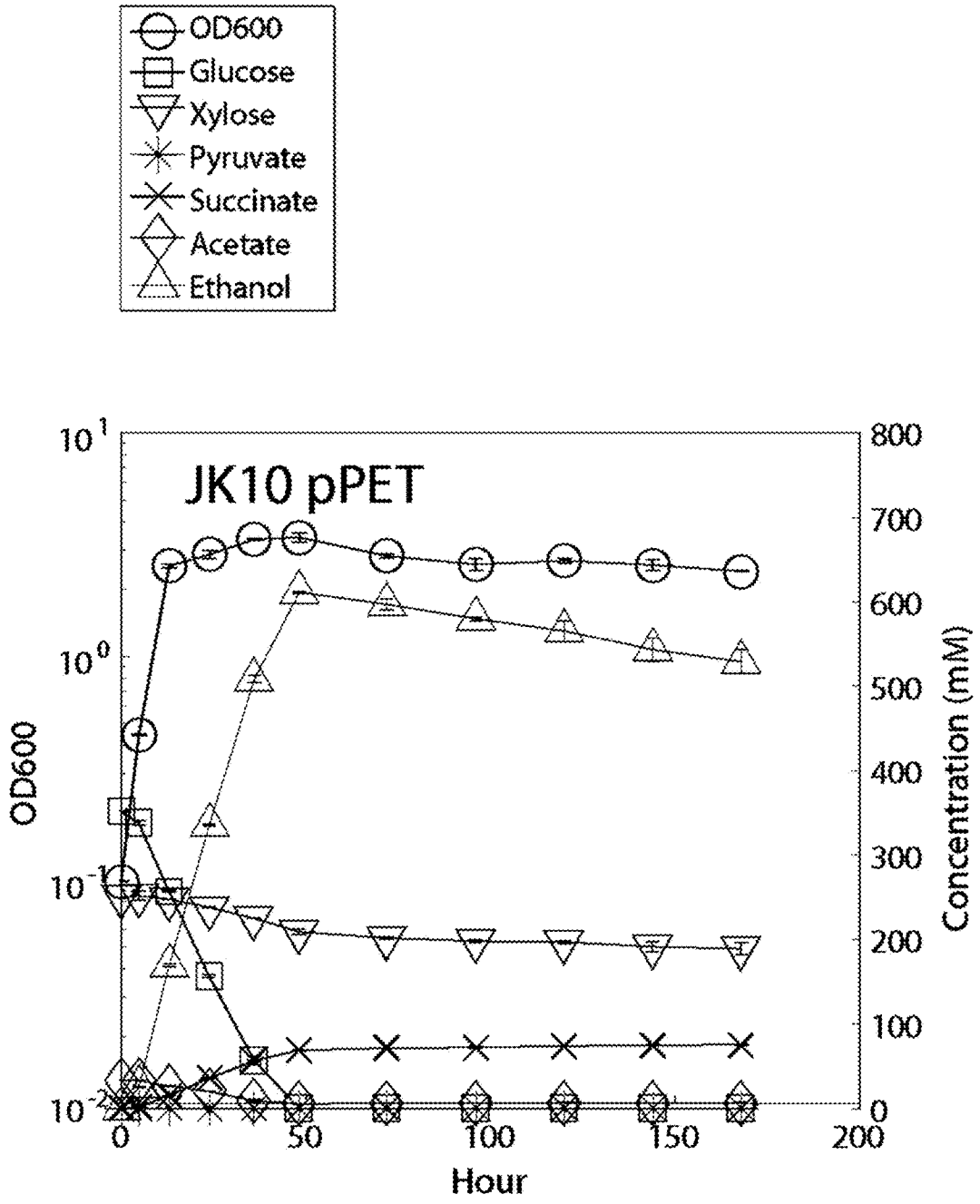
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
JK10 pPET
OD600
Concentration (mM)
Hour
10^1
10^0
10^{-1}
10^{-2}
800
700
600
500
400
300
200
100
0
0
50
100
150
200

FIG. 3E

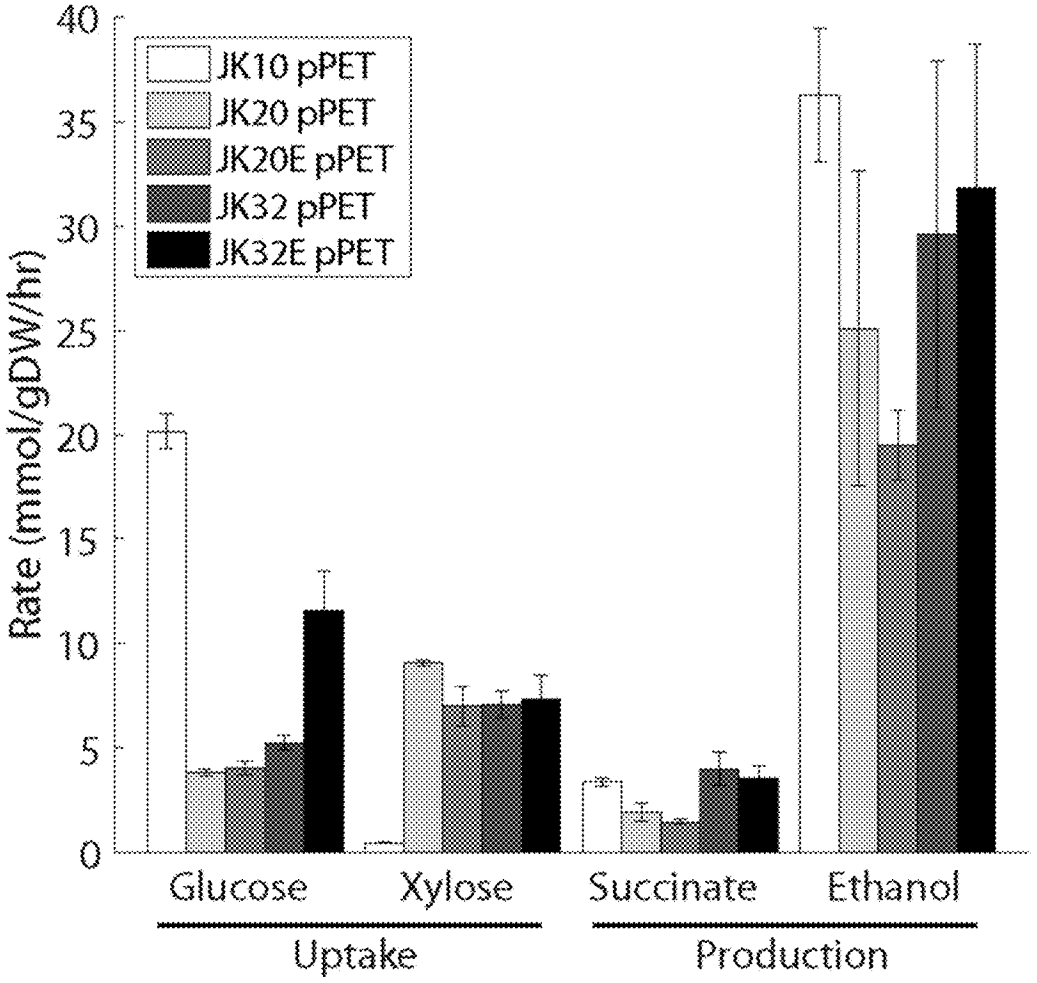
40
35
30
25
20
15
10
5
0
Rate (mmol/gDW/hr)
JK10 pPET
JK20 pPET
JK20E pPET
JK32 pPET
JK32E pPET
Glucose
Xylose
Succinate
Ethanol
Uptake
Production

FIG. 3F

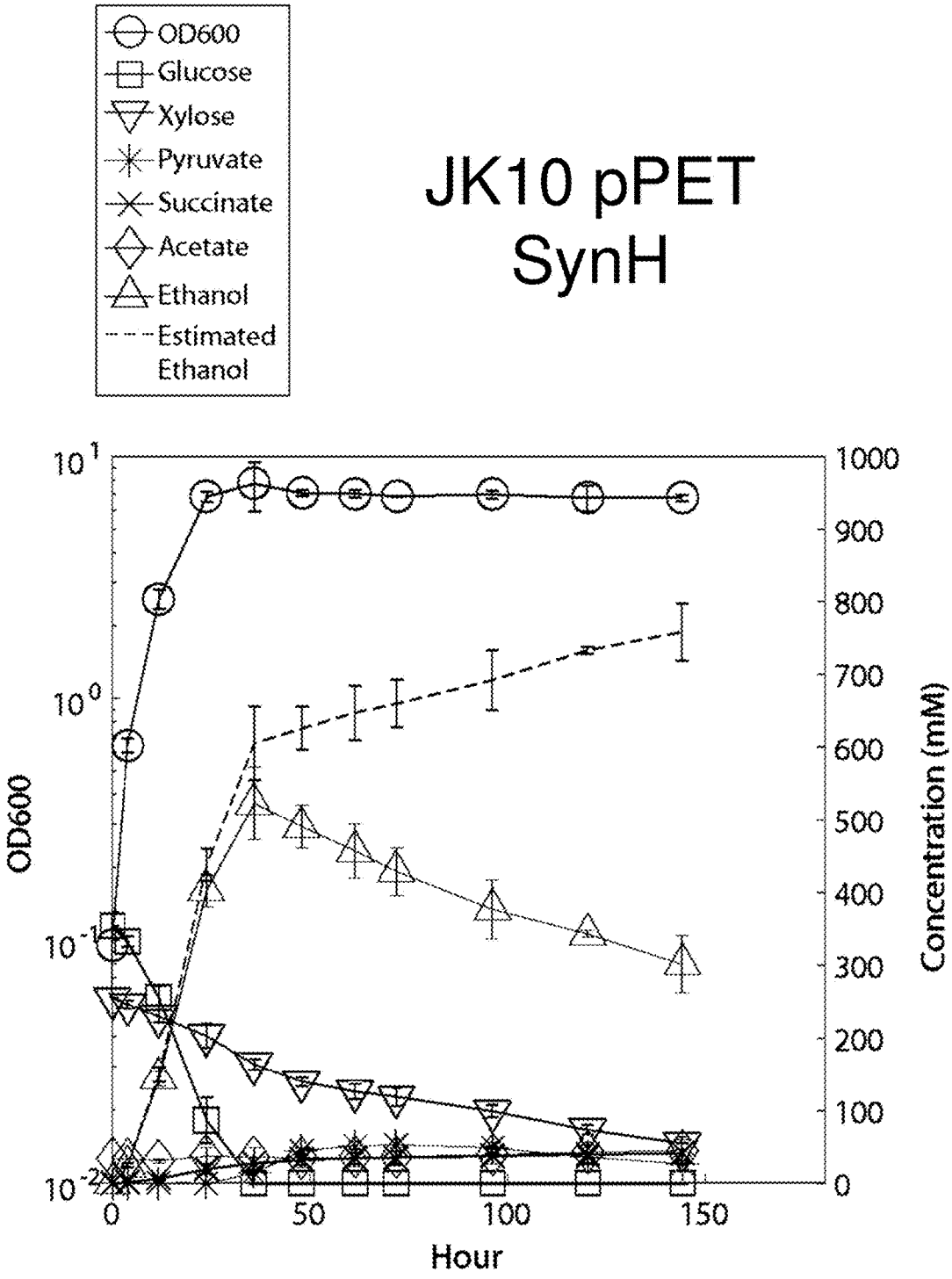
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK10 pPET SynH
OD600
Concentration (mM)
Hour
10^1
10^0
10^-1
10^-2
0
50
100
150
1000
900
800
700
600
500
400
300
200
100
0

FIG. 4A

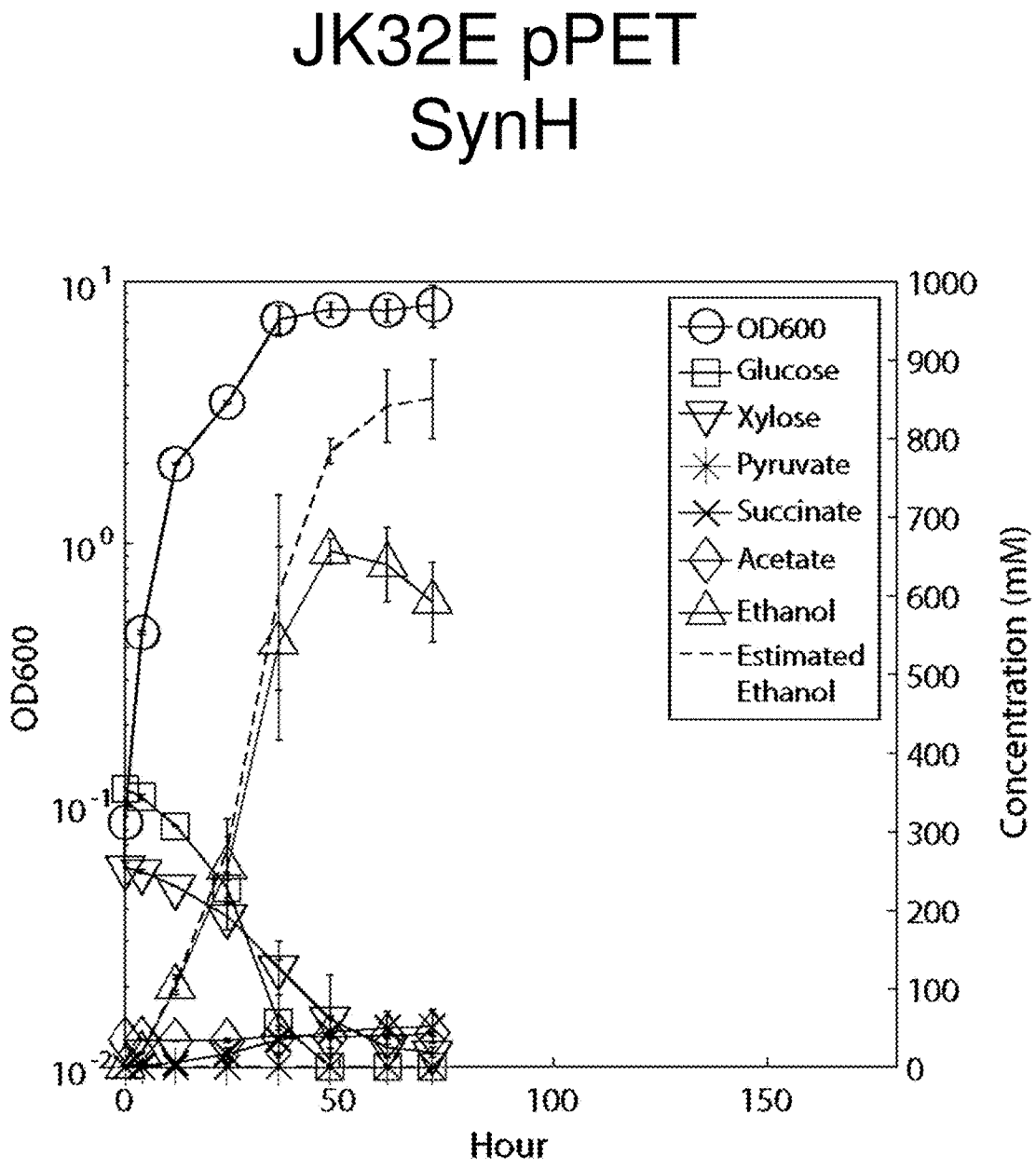
JK32E pPET
SynH
OD600
Concentration (mM)
Hour
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
10^{1}
10^{0}
10^{-1}
10^{-2}
0
50
100
150
1000
900
800
700
600
500
400
300
200
100
0

FIG. 4B

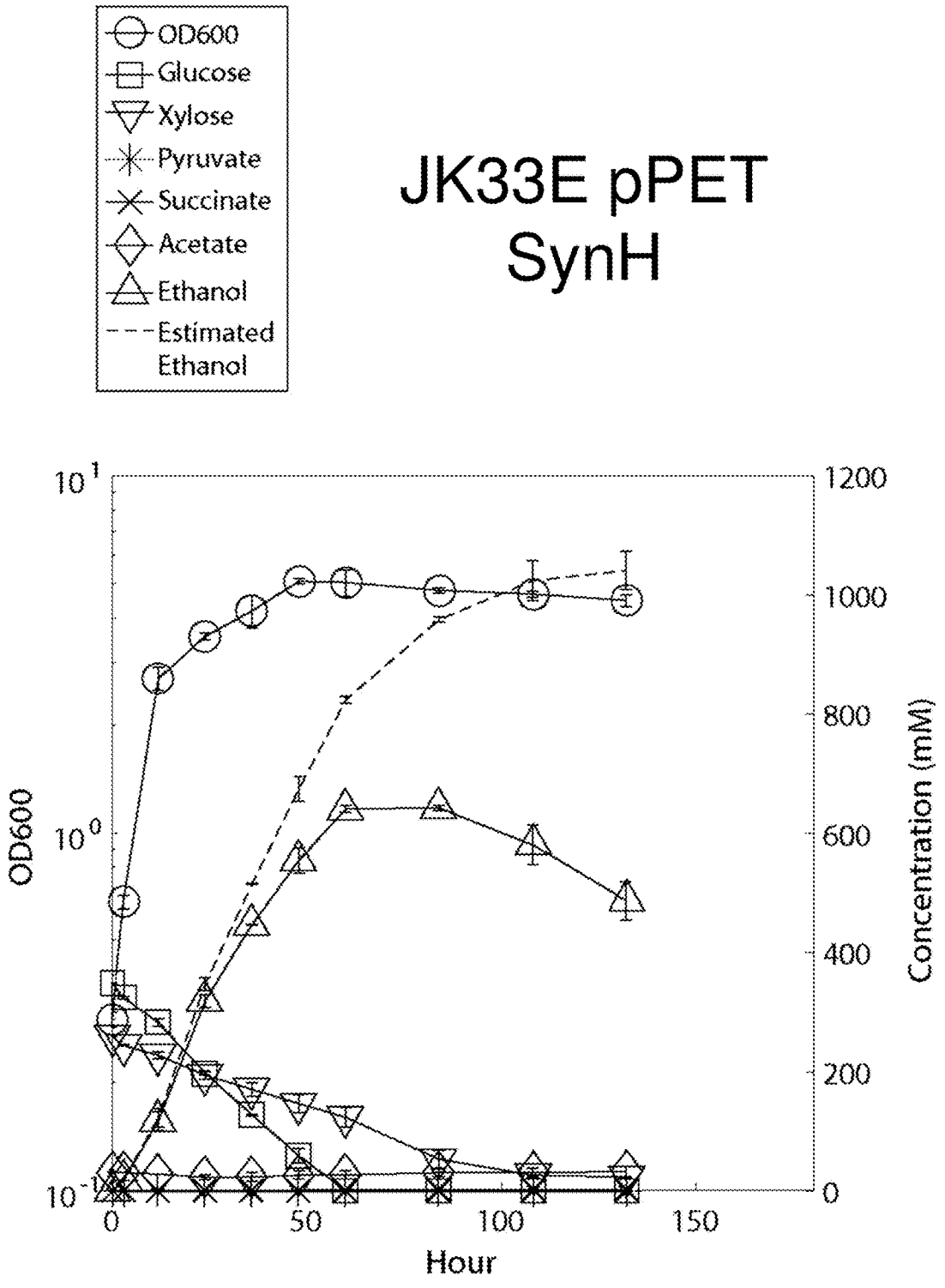
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK33E pPET SynH
10^1
10^0
10^{-1}
OD600
1200
1000
800
600
400
200
0
Concentration (mM)
0
50
100
150
Hour

FIG. 4C

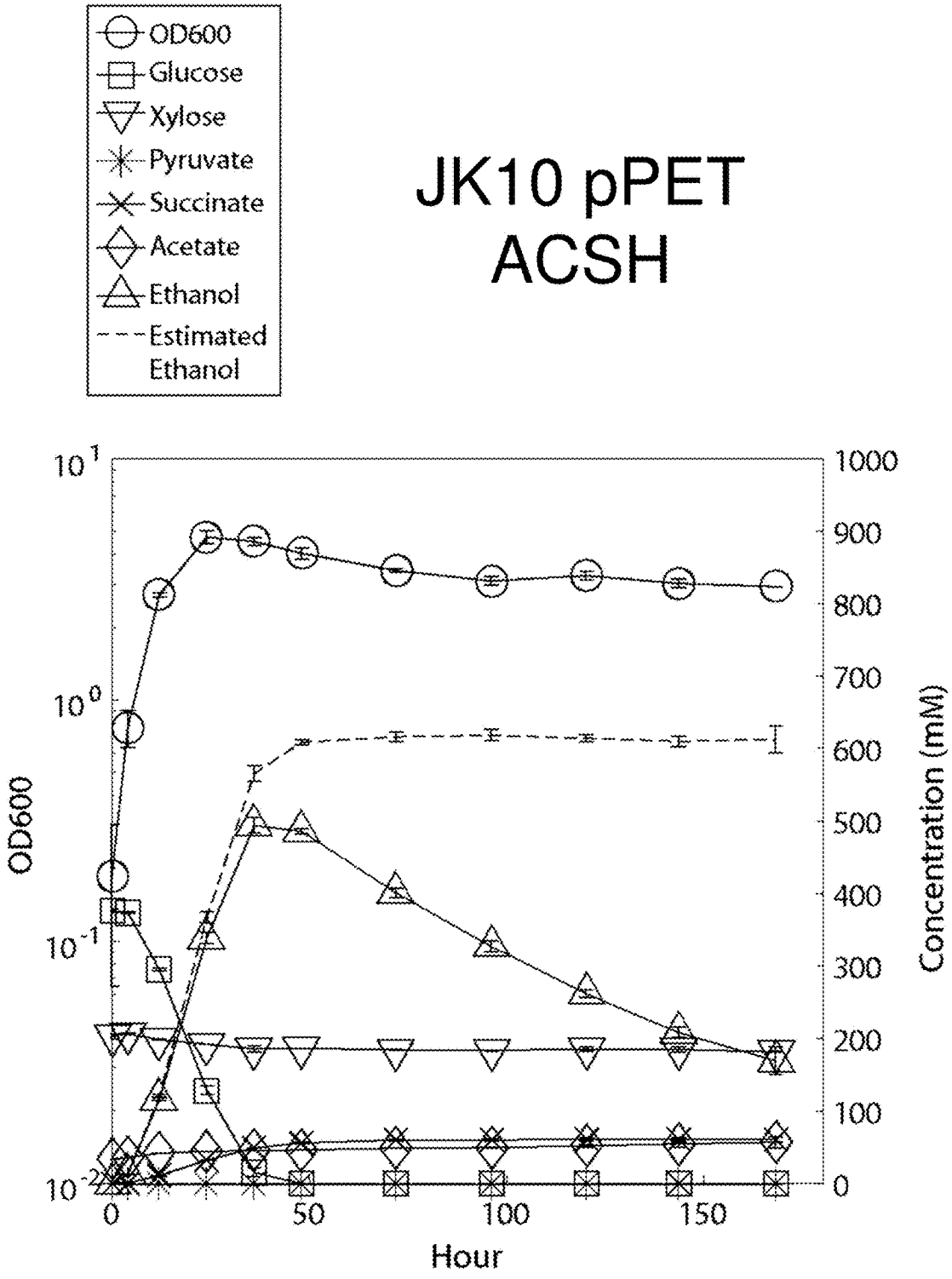
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK10 pPET ACSH
OD600
Concentration (mM)
Hour
10^1
10^0
10^-1
10^-2
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150

FIG. 4D

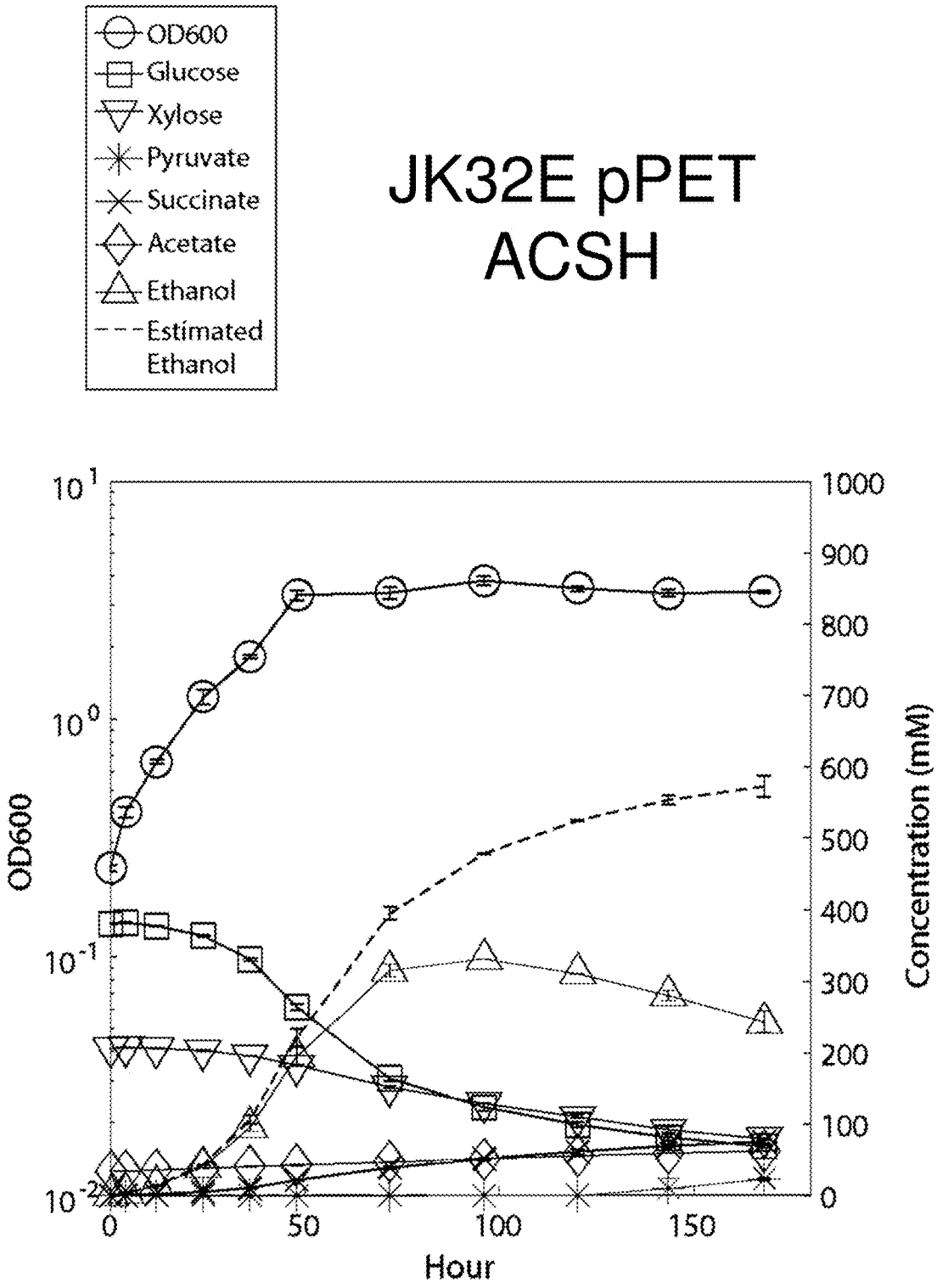
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK32E pPET ACSH
OD600
Concentration (mM)
Hour
10^{1}
10^{0}
10^{-1}
10^{-2}
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150

FIG. 4E

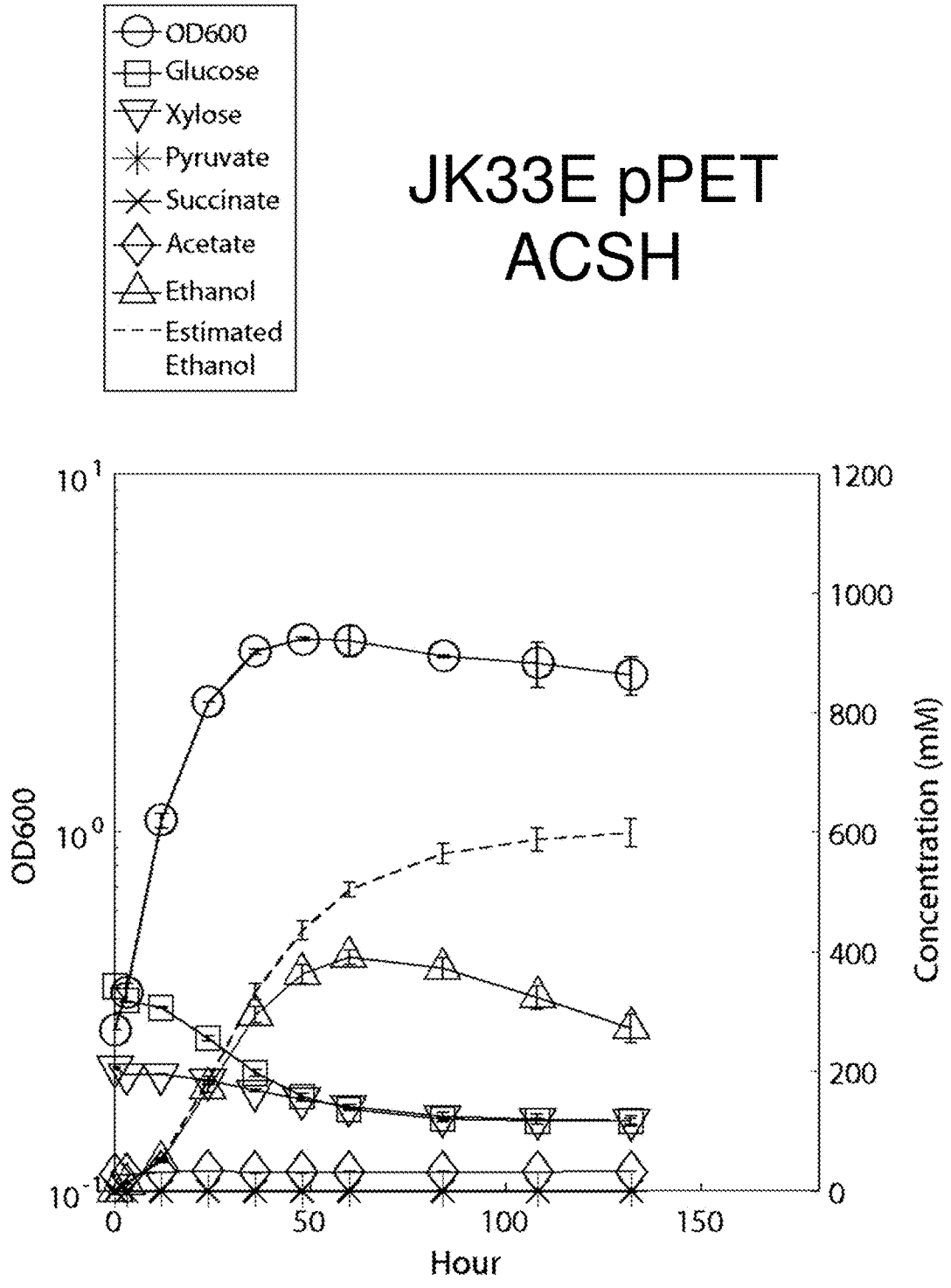
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK33E pPET ACSH
OD600
Concentration (mM)
Hour
10^1
10^0
10^-1
0
50
100
150
1200
1000
800
600
400
200
0

FIG. 4F

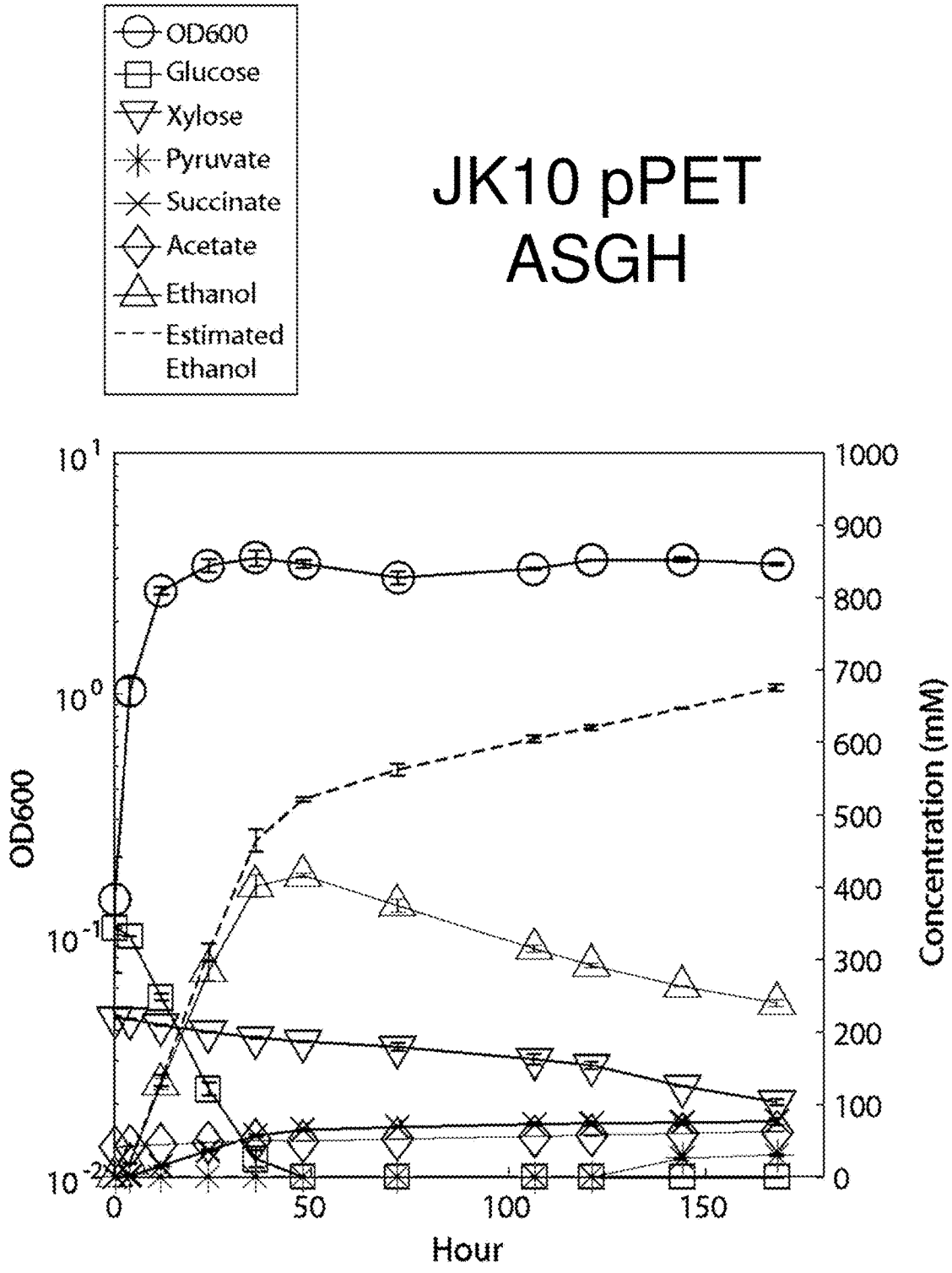
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK10 pPET ASGH
OD600
Concentration (mM)
Hour
10^{1}
10^{0}
10^{-1}
10^{-2}
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150

FIG. 4G

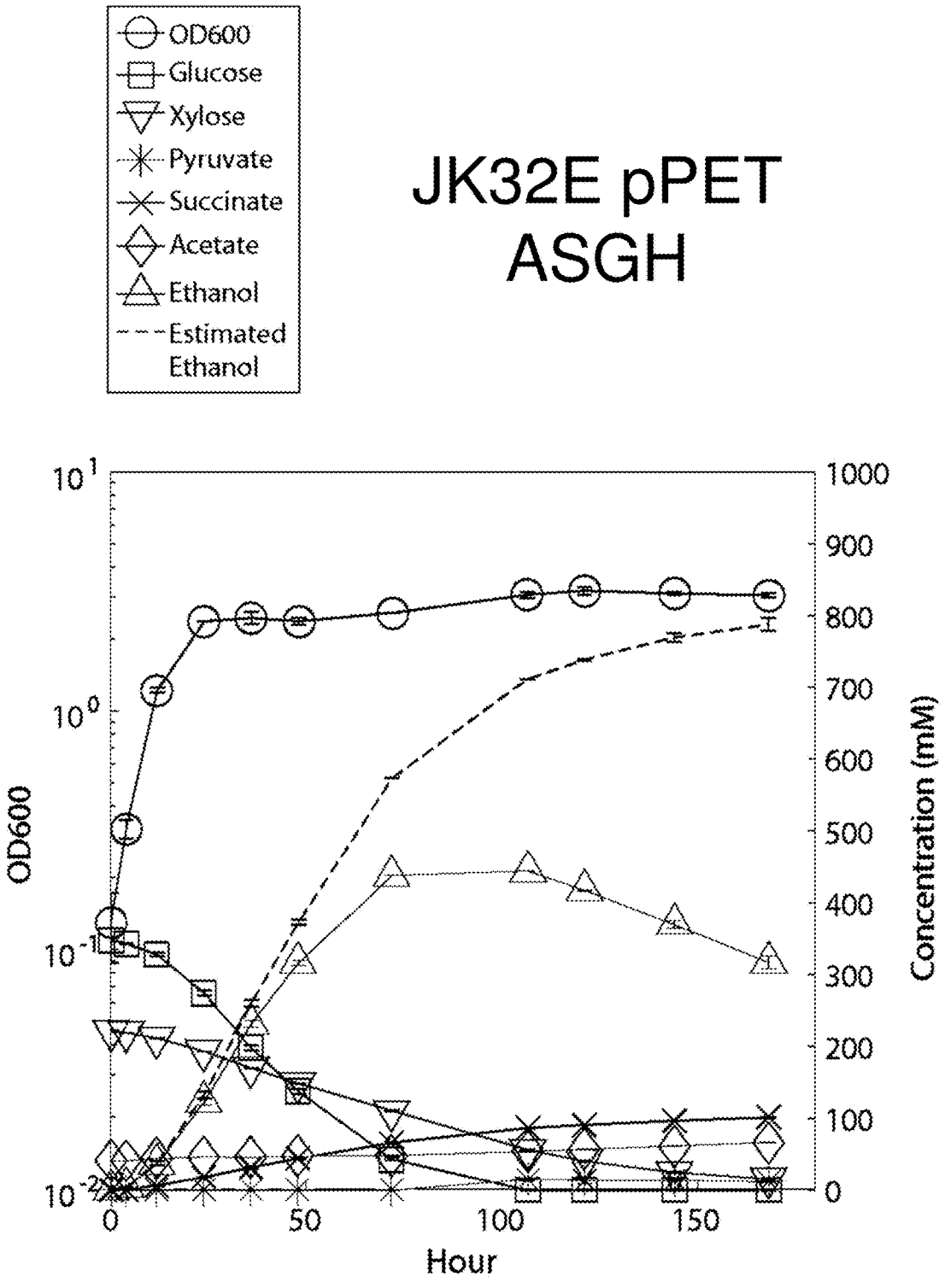
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK32E pPET ASGH
OD600
Concentration (mM)
Hour
10^1
10^0
10^-1
10^-2
1000
900
800
700
600
500
400
300
200
100
0
0
50
100
150

FIG. 4H

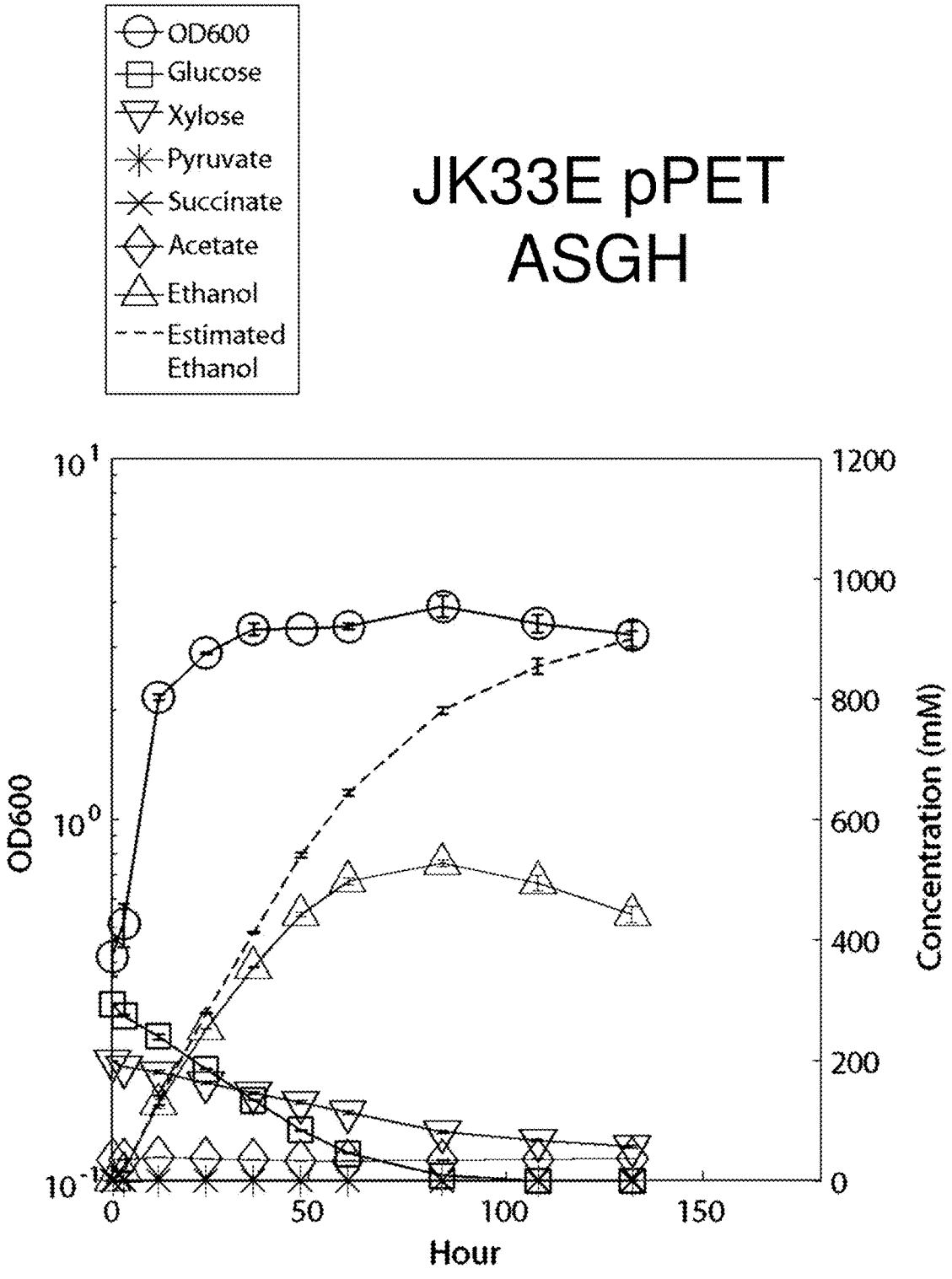
OD600
Glucose
Xylose
Pyruvate
Succinate
Acetate
Ethanol
Estimated Ethanol
JK33E pPET ASGH
10^1
10^0
10^{-1}
OD600
1200
1000
800
600
400
200
0
Concentration (mM)
0
50
100
150
Hour

FIG. 4I

MICROORGANISMS THAT CO-CONSUME GLUCOSE WITH NON-GLUCOSE CARBOHYDRATES AND METHODS OF USE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under DE-FC02-07ER64494 awarded by the US Department of Energy. The government has certain rights in the invention.

FIELD OF THE INVENTION

The invention is generally directed to microorganisms that co-consume glucose with non-glucose carbohydrates as well as methods of using the microorganisms for the production of commodity chemicals. The invention in a specific aspect is directed to microorganisms that co-consume glucose and xylose, e.g., in lignocellulosic hydrolysates, for converting the glucose and xylose into ethanol.

BACKGROUND

Ethanol obtained from the fermentation of starch from grains or sucrose from sugar cane is blended with gasoline to supplement petroleum supplies. The relatively oxygenated ethanol increases the efficiency of combustion and the octane value of the fuel mixture. Production of ethanol from grain and other foodstuffs, however, can limit the amount of agricultural land available for food and feed production, thereby raising the market prices of grains and leading to the expansion of agricultural production into forests or marginal lands thereby resulting in ecological damage. Moreover, the intense tillage and fertilization of prime agricultural land for the production of grains can result in excessive soil erosion and runoff or penetration of excess phosphorous and nitrogen into waterways and aquifers. Production of ethanol from lignocellulosic agricultural or woody feedstocks that do not compete with food and animal feed supplies is therefore highly desirous for the large-scale development of renewable fuels from biomass.

Several obstacles currently limit the use of biomass for renewable fuel production. The biomass must be pretreated to extract the sugars, lignins, and other components from the starting material. Mild conditions for pre-hydrolysis are desirable because they result in the formation of lower amounts of inhibitory components such as furfural, hydroxymethyl furfural, and sugar degradation products such as formic acid. The resulting sugars can be present in the form of monosaccharides such as D-glucose, D-xylose, D-mannose, D-galactose and L-arabinose or as various oligomers or polymers of these constituents along with other lignocellulosic components such as acetic acid, 4-O-methylglucuronic acid, and ferulic acid. Glucose in sugar hydrolysates may repress the induction of transcripts for proteins essential for the assimilation of less readily utilized sugars that are also present in hydrolysates, such as xylose, cellobiose, galactose, arabinose, and rhamnose. In addition, the production of ethanol from glucose can attain inhibitory concentrations even before use of other sugars commences. This results in the incomplete utilization of sugars and sugar mixtures in hydrolysates. Glucose in sugar hydrolysates may also repress the induction of transcripts for proteins essential for the depolymerization of cellulose, cellulo-oligosaccharides, xylan, xylo-oligosaccharides, mannan, manno-oligosaccharides, and other more complex hemicelluloses and oligosaccharides derived from them. These poly- and oligosaccharides can be present in hydrolysates that have been recovered under mild treatment conditions.

Bacteria such as *Escherichia coli, Zymomonas mobilis*, and *Klebsiella oxytoca* and yeasts such as *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* have been engineered for the production of ethanol from xylose, arabinose, xylo- and cellulo-oligosaccharides since native strains of these organisms are limited either by low production rates, strong preference for utilization of glucose over xylose, susceptibility to inhibitors, susceptibility to microbial or bacteriophage contamination, high requirements for nutrients, or containment regulations due to the expression of transgenes in order to achieve xylose or cellobiose utilization. There remains a need for microorganisms that will ferment glucose, xylose, and other sugars from lignocellulosic materials at high rates and yields without these drawbacks.

SUMMARY OF THE INVENTION

The invention provides microorganisms and uses thereof that address at least some of the above-mentioned needs.

The microorganisms of the invention include recombinant microorganisms. One version is a microorganism comprising modifications that reduce or ablate the activity of a phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) protein selected from the group consisting of an enzyme I (EI), an HPr, an FPr, and an enzyme II^{Glc} (EII^{Glc}). In some versions, the microorganism comprises a modification that reduces or ablates the activity of one or both of HPr and FPr. The HPr may comprise the HPr of *E. coli* or an ortholog thereof, and the FPr may comprise the FPr of *E. coli* or an ortholog thereof. In some versions, the microorganism comprises a modification that reduces or ablates the activity of one or both of an EI and an EII^{Glc}.

Another version is a microorganism comprising a first modification that reduces or ablates the activity of a phosphoglucose isomerase and a second modification selected from the group consisting of a modification that reduces or ablates the activity of GntR, a modification that introduces a recombinant phosphogluconate dehydratase gene, a modification that introduces a recombinant 2-keto-4-hydroxyglutarate aldolase gene, a modification that introduces a recombinant 2-keto-3-deoxy-6-phosphogluconate aldolase gene, and a modification that introduces a recombinant oxaloacetate decarboxylase gene. In some versions, the second modification comprises a modification that reduces or ablates the activity of GntR. In some versions, the second modification comprises a modification that introduces a recombinant phosphogluconate dehydratase gene. In some versions, the second modification comprises one or more modifications that introduce one or more of a recombinant 2-keto-4-hydroxyglutarate aldolase gene, a recombinant 2-keto-3-deoxy-6-phosphogluconate aldolase gene, and a recombinant oxaloacetate decarboxylase gene. In some versions, the second modification comprises one or more modifications that introduce a recombinant phosphogluconate dehydratase gene and one or more of a recombinant 2-keto-4-hydroxyglutarate aldolase gene, a recombinant 2-keto-3-deoxy-6-phosphogluconate aldolase gene, and a recombinant oxaloacetate decarboxylase gene. In some versions, the second modification comprises one or more modifications that introduce a recombinant phosphogluconate dehydratase gene, a recombinant 2-keto-4-hydroxyglutarate aldolase gene, a recombinant 2-keto-3-deoxy-6-phosphogluconate aldolase gene, and a recombinant oxaloacetate decarboxylase gene.

The microorganisms in any of the above-mentioned versions may further comprise at least one, some, or all of a modification that reduces or ablates the activity of a pyruvate formate lyase, a modification that reduces or ablates the activity of a lactate dehydrogenase, a modification that reduces or ablates the activity of a fumarate reductase, a modification that introduces a recombinant pyruvate decarboxylase gene, and a modification that introduces a recombinant alcohol dehydrogenase gene.

A method of the invention comprises consuming a carbohydrate by culturing a microorganism as described herein in a medium. The medium preferably comprises glucose and xylose, and the culturing preferably co-consumes the xylose with the glucose. The medium may comprise a biomass hydrolysate, such as an enzymatic or acid hydrolysate. In some versions, the microorganism is adapted to growth in a first medium comprising a component selected from the group consisting of glucose, xylose, and ethanol prior to culturing the microorganism in the medium. In some versions, the culturing produces an amount of ethanol during the consumption of the carbohydrate.

The objects and advantages of the invention will appear more fully from the following detailed description of the preferred embodiment of the invention made in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a schema of the development and fermentation characteristics of exemplary microorganisms of the invention.

FIGS. **2**A-**2**D show growth (OD600) and compound concentrations versus time for microorganisms of the invention cultured anaerobically in a minimal medium containing glucose and xylose.

FIGS. **3**A-**3**E show growth (OD600) and compound concentrations versus time for microorganisms of the invention cultured anaerobically in shake flasks in a synthetic hydrolysate medium (SynH, which lacks lignotoxins). FIG. **3**F shows glucose and xylose uptake rates and succinate and ethanol production rates for microorganisms of the invention.

FIGS. **4**A-**4**I show growth (OD600) and compound concentrations versus time for microorganisms of the invention cultured anaerobically in a bioreactor with a synthetic hydrolysate medium (SynH) (FIGS. **4**A-**4**C), ammonia fiber explosion (AFEX)-pretreated corn stover hydrolysate (ACSH) (FIGS. **4**D-**4**F), or AFEX-pretreated switchgrass hydrolysate (ASGH) (FIGS. **4**G-**4**I).

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the invention is a recombinant (i.e., genetically modified) microorganism. The microorganism may be a eukaryotic microorganism or a prokaryotic microorganism. Exemplary eukaryotic microorganisms include protists and yeasts. Exemplary prokaryotes include bacteria and archaea. Examples of suitable bacterial cells include gram-positive bacteria such as strains of *Bacillus*, (e.g., *B. brevis* or *B. subtilis*), *Pseudomonas*, and *Streptomyces*, and gram-negative bacteria, such as strains of *Escherichia coli* and *Aeromonas hydrophila*. Examples of suitable yeast cells include strains of *Saccharomyces* (e.g., *S. cerevisiae*), *Schizosaccharomyces, Kluyveromyces, Pichia* (e.g., *P. pastoris* or *P. methlanolica*), *Hansenula* (e.g., *H. Polymorpha*), *Yarrowia, Scheffersomyces*, (e.g., *S. stipitis*), and *Candida*.

In some versions of the invention, the microorganism is a microorganism that comprises a phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS). The PTS is a well characterized carbohydrate transport system utilized by microorganisms such as bacteria. See Postma et al. 1993 and Tchieu et al. 2001, which are incorporated herein by reference in their entirely. Exemplary bacteria comprising the PTS include those from the genera *Bacillus, Clostridium, Enterobacteriaceae, Enterococcus, Erwinia, Escherichia, Klebsiella, Lactobacillus, Lactococcus, Mycoplasma, Pasteurella, Rhodobacter, Rhodoseudomonas, Salmonella, Staphylococcus, Streptococcus, Vibrio*, and *Xanthomonas*. Exemplary species include *E. coli, Salmonella typhimurium, Staphylococcus camosus, Bacillus subtilis, Mycoplasma capricolum, Enterococcus faecalis, Staphylococcus aureus, Streptococcus salivarius, Streptococcus mutans, Klebsiella pneumoniae, Staphylococcus carnosus, Streptococcus sanguis, Rhodobacter capsulatus, Vibrio alginolyticus, Erwinia chrysanthemi, Xanthomonas campestris, Lactococcus lactis, Lactobacillus casei, Rhodoseudomonas sphaeroides, Erwinia carotovora, Pasteurella multocida*, and *Clostridium acetobutylicum*.

In some versions of the invention, the microorganism comprises intact fadA, fadB, fadI, fadJ, and/or fadR genes or expresses the functional gene products thereof.

The microorganisms of the invention comprise modifications that reduce or ablate the activity of gene products of one or more genes. Such a modification that that reduces or ablates the activity of gene products of one or more genes is referred to herein as a "functional deletion" of the gene product. "Functional deletion" or its grammatical equivalents refers to any modification to a microorganism that ablates, reduces, inhibits, or otherwise disrupts production of a gene product, renders a produced gene product non-functional, or otherwise reduces or ablates a produced gene product's activity. Accordingly, in some instances, a gene product that is functionally deleted means that the gene product is not produced by the microorganism at all. "Gene product" refers to a protein or polypeptide encoded and produced by a particular gene. "Gene" refers to a nucleic acid sequence capable of producing a gene product and may include such genetic elements as a coding sequence together with any other genetic elements required for transcription and/or translation of the coding sequence. Such genetic elements may include a promoter, an enhancer, and/or a ribosome binding site (RBS), among others.

One of ordinary skill in the art will appreciate that there are many well-known ways to functionally delete a gene product. For example, functional deletion can be accomplished by introducing one or more genetic modifications. As used herein, "genetic modifications" refer to any differences in the nucleic acid composition of a cell, whether in the cell's native chromosome or in endogenous or exogenous non-chromosomal plasmids harbored within the cell. Examples of genetic modifications that may result in a functionally deleted gene product include but are not limited to substitutions, partial or complete deletions, insertions, or other variations to a coding sequence or a sequence controlling the transcription or translation of a coding sequence, such as placing a coding sequence under the control of a less active promoter, etc. In some versions, a gene or coding sequence can be replaced with a selection marker or screenable marker. Various methods for introducing genetic modifications are well known in the art and include homologous recombination, among other mechanisms. See, e.g., Green et al., *Molecular Cloning: A laboratory manual*, 4th ed., Cold Spring Harbor Laboratory Press (2012) and Sambrook et al.,

Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press (2001). Various other genetic modifications that functionally delete a gene product are described in the examples below. In some versions, functional deletion can be accomplished by expressing ribozymes or antisense sequences that target the mRNA of the gene of interest. Functional deletion can also be accomplished by inhibiting the activity of the gene product, for example, by chemically inhibiting a gene product with a small-molecule inhibitor, by expressing a protein that interferes with the activity of the gene product, or by other means.

In certain versions of the invention, the functionally deleted gene product may have less than about 95%, less than about 90%, less than about 85%, less than about 80%, less than about 75%, less than about 70%, less than about 65%, less than about 60%, less than about 55%, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or about 0% of the activity of the non-functionally deleted gene product.

In certain versions of the invention, a cell with a functionally deleted gene product may have less than about 95%, less than about 90%, less than about 85%, less than about 80%, less than about 75%, less than about 70%, less than about 65%, less than about 60%, less than about 55%, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or about 0% of the activity of the gene product compared to a cell with the non-functionally deleted gene product.

In certain versions of the invention, the functionally deleted gene product may be expressed at an amount less than about 95%, less than about 90%, less than about 85%, less than about 80%, less than about 75%, less than about 70%, less than about 65%, less than about 60%, less than about 55%, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or about 0% of the amount of the non-functionally deleted gene product.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, or more nonsynonymous substitutions are present in the gene or coding sequence of the gene product.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, or more bases are inserted in the gene or coding sequence of the gene product.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of the gene product's gene or coding sequence is deleted or mutated.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of a promoter driving expression of the gene product is deleted or mutated.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of an enhancer controlling transcription of the gene product's gene is deleted or mutated.

In certain versions of the invention, the functionally deleted gene product may result from a genetic modification in which at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% of a sequence controlling translation of the gene product's mRNA is deleted or mutated.

In certain versions of the invention, the decreased activity or expression of the functionally deleted gene product is determined with respect to the activity or expression of the gene product in its unaltered state as found in nature. In certain versions of the invention, the decreased activity or expression of the functionally deleted gene product is determined with respect to the activity or expression of the gene product in its form in a corresponding microorganism. In certain versions, the genetic modifications giving rise to a functionally deleted gene product are determined with respect to the gene in its unaltered state as found in nature. In certain versions, the genetic modifications giving rise to a functionally deleted gene product are determined with respect to the gene in its form in a corresponding microorganism. As used herein, "corresponding microorganism" refers to a microorganism of the same species having the same or substantially same genetic and proteomic composition as a microorganism of the invention, with the exception of genetic and proteomic differences resulting from the manipulations described herein for the microorganisms of the invention.

In some versions of the invention, a gene product of the PTS is functionally deleted. The PTS may comprise such proteins as enzyme I (EI), HPr, FPr, multiphosphoryl transfer protein (MTP), enzyme II (EII) and enzyme III (EIII). The following reactions comprise exemplary PTS-mediated translocation and phosphorylation reactions with a given carbohydrate (P=phospho group):

$$\text{P-enolpyruvate+EI} \leftrightarrow \text{P-EI+pyruvate} \quad (1)$$

$$\text{P-EI+HPr} \leftrightarrow \text{P-HPr+EI} \quad (2)$$

$$\text{P-HPr+EIIA (domain or protein)} \leftrightarrow \text{P-EIIA+HPr} \quad (3)$$

P-EIIA+EIIB (domain or protein)↔P-EIIB+EIIA (4)

EIIC

P-EIIB+carbohydrate$_{(out)}$→EIIB+carbohydrate-P$_{(in)}$ (5)

In most cases, EI and HPr are soluble, cytoplasmic proteins that participate in the phosphorylation of all PTS carbohydrates in a given organism and thus have been called the general PTS proteins. The EIIs are carbohydrate specific and may consist of a single membrane-bound protein composed of three domains (A, B, and C), such as that for mannitol (EII^{Mtl}) in *E. coli*, or of two or more proteins, at least one of which is membrane bound (e.g., B and C) and one of which is soluble (IIA or EIII), such as the $IICB^{glc}$-IIA^{Glc} pair for glucose in *E. coli*. In both cases, the phospho group is transferred from PEP to the incoming carbohydrate via obligatory phospho intermediates of EI, HPr, EIIA, and EIIB. The EIIC domain, which makes up the integral membrane portion of an EII, presumably forms its translocation channel and at least part of its specific substrate-binding site. In a third variation, exemplified by the mannose PTS in *E. coli*, both A and B domains are fused in a single soluble polypeptide, while there are two integral membrane proteins (IIC^{Man} and IID^{Man}) involved in mannose translocation.

Although the glucose, mannitol, and mannose PTSs of *E. coli* are the most common in terms of protein organization in most organisms, other variations are possible. For example, the cellobiose PTS in *E. coli* has been shown to have each functional domain of its EII as a separate protein: two soluble proteins (IIA^{Cel} and IIB^{Cel}) that each contain a site of covalent phosphorylation, and one membrane-bound protein (IIC^{Cel}). For PTS-mediated fructose transport, a protein called FPr (fructose-specific HPr) combines the functions of the IIA^{Fru} domain and of an HPr-like protein (Geerse et al. 1989). In this case, then, it is FPr rather than HPr that is phosphorylated by EI (on the HPr-like domain) as an intermediate in fructose translocation.

In some versions of the invention, an EI in the recombinant microorganism is functionally deleted. EIs include enzymes classified under Enzyme Commission (EC) number 2.7.3.9. An exemplary EI is the PtsI of *E. coli*, which is encoded by ptsI. An exemplary sequence of the *E. coli* PtsI is SEQ ID NO:2, and an exemplary sequence of the *E. coli* ptsI is SEQ ID NO:1. Other EIs include homologs of the *E. coli* PtsI. Homologs of the *E. coli* PtsI include orthologs of the *E. coli* PtsI, paralogs of such orthologs having PtsI activity, and paralogs of the *E. coli* PtsI in *E. coli* having PtsI activity. The *E. coli* PtsI and homologs thereof are well-recognized in the art. See Postma et al. 1993, Tchieu et al. 2001, Robillard et al. 1979, Waygood et al. 1980, Byrne et al 1988, De Reuse et al. 1988, Saffen et al. 1987, Weigel et al. 1982, LiCalsi et al. 1991, Schnierow et al. 1989, Kohlbrecher et al. 1992, Reizer et al. 1992, Gonzy-Tréboul et al. 1989, Jaffor et al. 1977, Alpert et al. 1985, Hengstenberg et al. 1976, Albert et al. 1985, Vadeboncoeur et al. 1983, Gagnon et al. 1992, and Thibault et al. 1985.

In some versions of the invention, an HPr in the recombinant microorganism is functionally deleted. HPrs include enzymes classified under EC 2.7.11.-. HPrs also include proteins classified as TC 8.A.8.1.1 in the Transporter Classification System. An exemplary HPr is the HPr of *E. coli*, which is encoded by ptsH. An exemplary sequence of the *E. coli* HPr is SEQ ID NO:4, and an exemplary sequence of the *E. coli* ptsH is SEQ ID NO:3. Other HPrs include homologs of the *E. coli* HPr. Homologs of the *E. coli* HPr include orthologs of the *E. coli* HPr, paralogs of such orthologs having the HPr activity, and paralogs of the *E. coli* HPr in *E. coli* having HPr activity. The *E. coli* HPr and homologs thereof are well-recognized in the art. See Postma et al. 1993, Tchieu et al. 2001 Anderson et al. 1971, Dooijewaard et al. 1979, Byrne et al. 1988, De Reuse et al. 1988, Saffen et al. 1987, Weigel et al. 1982, Beneski et al. 1982, Byrne et al. 1988, Powers et al. 1984, Schnierow et al. 1989, Titgemeyer et al. 1990, Kalbitzer et al. 1982, Marquet et al. 1976, Reizer et al. 1989, Reizer et al. 1989, Gonzy-Tréboul et al. 1989, Jaffor et al. 1976, Beyreuther et al. 1977, Simoni et al. 1973, Reizer et al. 1988, Eisermann et al. 1991, Deutscher et al. 1986, Vadeboncoeur et al. 1983, Waygood et al. 1986, Mimurs et al. 1984, Thibault et al. 1985, and Jenkinson 1989.

In some versions of the invention, an FPr in the recombinant microorganism is functionally deleted. FPrs include enzymes classified under EC 2.7.1.69. An exemplary FPr is the FPr of *E. coli*, which is encoded by fruB. An exemplary sequence of the *E. coli* FPr is SEQ ID NO:6, and an exemplary sequence of the *E. coli* fruB is SEQ ID NO:5. Other FPrs include homologs of the *E. coli* FPr. Homologs of the *E. coli* FPr include orthologs of the *E. coli* FPr, paralogs of such orthologs having FPr activity, and paralogs of the *E. coli* FPr in *E. coli* having FPr activity. The *E. coli* FPr and homologs thereof are well-recognized in the art. See Postma et al. 1993, Tchieu et al. 2001, Waygood 1980, Geerse et al. 1986, Sutrina et al. 1988, and Geerse et al. 1989.

In some versions of the invention, a glucose-specific EII (EII^{Glc}) is functionally deleted. EII^{Glc} proteins include any protein comprising one or more of an EIIA domain, an EIIB domain, and an EIIC domain having activity for glucose. $EIIA^{Glc}$ domains include those having activity classified under EC:2.7.1.-. $EIIB^{Glc}$ domains include those having activity classified under EC:2.7.1.69. An exemplary EII^{Glc} is the $EIIA^{Glc}$ of *E. coli*, which is encoded by crr. An exemplary sequence of the *E. coli* IIA^{Glc} is SEQ ID NO:8, and an exemplary sequence of the *E. coli* crr is SEQ ID NO:7. Another exemplary EII^{Glc} is the PTS system glucose-specific EIICB component (PTGCB) of *E. coli*, which is encoded by ptsG. An exemplary sequence of the *E. coli* PTGCB is SEQ ID NO:10, and an exemplary sequence of the *E. coli* ptsG is SEQ ID NO:9. EII^{Glc} proteins are well-recognized in the art. See Postma et al. 1993, Tchieu et al. 2001, Erni et al. 1986, Saffen et al. 1987, Nelson et al. 1984, Gonzy-Tréboul et al. 1991, Gonzy-Tréboul et al. 1989, Zagorec et al. 1992, Reidl et al. 1991, Boos et al. 1990, Peri et al. 1990, Peri et al. 1988, Rogers et al. 1988, Vogler et al. 1991, Ebner et al. 1988, Lengeler et al. 1992, Blatch et al. 1990, Fouet et al. 1987, Zukowski et al. 1990, Sato et al. 1989, Bramley et al. 1987, Schnetz et al. 1987, El Hassouni et al. 1992, and Hall et al. 1992.

In some versions of the invention, a non-PTS protein is functionally deleted. Microorganisms in which non-PTS proteins are functionally deleted may or may not comprise a PTS and may comprise any type of microorganism described herein.

Accordingly, in some versions of the invention a phosphoglucose isomerase in the recombinant microorganism is functionally deleted. Phosphoglucose isomerases are also known as glucose-6-phosphate isomerases and phosphohexose isomerases. Phosphoglucose isomerases include enzymes classified under EC 5.3.1.9. An exemplary phosphoglucose isomerase is the glucose-6-phosphate isomerase of *E. coli*, which is encoded by pgi. An exemplary sequence of the *E. coli* glucose-6-phosphate isomerase is SEQ ID NO:12, and an exemplary sequence of the *E. coli* pgi is SEQ ID NO:11. Other phosphoglucose isomerases include

homologs of the *E. coli* glucose-6-phosphate isomerase. Homologs of the *E. coli* glucose-6-phosphate isomerase include orthologs of the *E. coli* glucose-6-phosphate isomerase, paralogs of such orthologs having phosphoglucose isomerase activity, and paralogs of the *E. coli* glucose-6-phosphate isomerase in *E. coli* having phosphoglucose isomerase activity. Phosphoglucose isomerases are well-recognized in the art.

In some versions of the invention a GntR in the recombinant microorganism is functionally deleted. GntRs are transcriptional regulators of enzymes involved in gluconate metabolism. An exemplary GntR is the GntR of *E. coli*, which is encoded by gntR. An exemplary sequence of the *E. coli* GntR is SEQ ID NO:14, and an exemplary sequence of the *E. coli* gntR is SEQ ID NO:13. Other GntRs include homologs of the *E. coli* GntR. Homologs of the *E. coli* GntR include orthologs of the *E. coli* GntR, paralogs of such orthologs having GntR activity, and paralogs of the *E. coli* GntR in *E. coli* having GntR activity. GntRs are well-recognized in the art. The deletion of GntR in *E. coli* is believed to lead to higher expression of the endogenous edd-eda operon, the latter of which encodes a phosphogluconate dehydratase and a multifunctional 2-keto-4-hydroxyglutarate aldolase/2-keto-3-deoxy-6-phosphogluconate aldolase. Thus, an additional or alternative modification to functionally deleting a GntR is expressing or overexpressing a phosphogluconate dehydratase, a 2-keto-4-hydroxyglutarate aldolase, a 2-keto-3-deoxy-6-phosphogluconate aldolase, and/or an oxaloacetate decarboxylase. Phosphogluconate dehydratases, 2-keto-4-hydroxyglutarate aldolases, 2-keto-3-deoxy-6-phosphogluconate aldolases, and oxaloacetate decarboxylases are discussed below.

In some versions of the invention, a lactate dehydrogenase in the recombinant microorganism is functionally deleted. Lactate dehydrogenases include enzymes classified under EC 1.1.1.27. An exemplary lactate dehydrogenase is the LdhA of *E. coli*, which is encoded by ldhA. An exemplary sequence of the *E. coli* LdhA is SEQ ID NO:16, and an exemplary sequence of the *E. coli* ldhA is SEQ ID NO:15. Other lactate dehydrogenases include homologs of the *E. coli* LdhA. Homologs of the *E. coli* LdhA include orthologs of the *E. coli* LdhA, paralogs of such orthologs having lactate dehydrogenase activity, and paralogs of the *E. coli* LdhA in *E. coli* having lactate dehydrogenase activity. The *E. coli* LdhA and homologs thereof are well-recognized in the art.

In some versions of the invention, a pyruvate formate lyase in the recombinant microorganism is functionally deleted. Pyruvate formate lyases include enzymes classified under EC 2.3.1.54. An exemplary pyruvate formate lyase is the PFL of *E. coli*, which is encoded by pflB. An exemplary sequence of the *E. coli* PFL is SEQ ID NO:18, and an exemplary sequence of the *E. coli* pflB is SEQ ID NO:17. Other pyruvate formate lyases include homologs of the *E. coli* PFL. Homologs of the *E. coli* PFL include orthologs of the *E. coli* PFL, paralogs of such orthologs having pyruvate formate lyase activity, and paralogs of the *E. coli* PFL in *E. coli* having pyruvate formate lyase activity. The *E. coli* PFL and homologs thereof are well-recognized in the art.

In some versions of the invention, a pyruvate formate lyase activating enzyme in the recombinant microorganism is functionally deleted. Pyruvate formate lyase activating enzymes include enzymes classified under EC 1.97.1.4. Pyruvate formate lyase activating enzymes activate pyruvate formate lyases. Functionally deleting a pyruvate formate lyase activating enzyme constitutes a way to functionally delete a pyruvate formate lyase. An exemplary pyruvate formate lyase activating enzyme is the PFL activase of *E. coli*, which is encoded by pflA. An exemplary sequence of the *E. coli* PFL activase is SEQ ID NO:20, and an exemplary sequence of the *E. coli* pflA is SEQ ID NO:19. Other pyruvate formate lyase activating enzymes include homologs of the *E. coli* PFL activase. Homologs of the *E. coli* PFL activase include orthologs of the *E. coli* PFL activase, paralogs of such orthologs having pyruvate formate lyase activating activity, and paralogs of the *E. coli* PFL activase in *E. coli* having pyruvate formate lyase activating activity. The *E. coli* PFL activase and homologs thereof are well-recognized in the art.

In some versions of the invention, fumarate reductase in the recombinant microorganism is functionally deleted. Fumarate reductases include enzymes classified under EC 1.3.5.4 and EC 1.3.1.6. Fumarate reductases are multisubunit enzymes, which typically comprise A, B, C, and D subunits. A fumarate reductase can be functionally deleted by, e.g., modifying the genes for any one or more of the subunits. An exemplary fumarate reductase is the QFR of *E. coli*, which is comprised of FrdA, FrdB, FrdC, and FrdD. FrdA is encoded by frdA. FrdB is encoded by frdB. FrdC is encoded by frdC. FrdD is encoded by frdD. An exemplary sequence of the *E. coli* FrdA is SEQ ID NO:22, and an exemplary sequence of the *E. coli* frdA is SEQ ID NO:21. An exemplary sequence of the *E. coli* FrdB is SEQ ID NO:24, and an exemplary sequence of the *E. coli* frdB is SEQ ID NO:23. An exemplary sequence of the *E. coli* FrdC is SEQ ID NO:26, and an exemplary sequence of the *E. coli* frdC is SEQ ID NO:25. An exemplary sequence of the *E. coli* FrdD is SEQ ID NO:28, and an exemplary sequence of the *E. coli* frdD is SEQ ID NO:27. Other fumarate reductases include homologs of the *E. coli* QFR. Homologs of the *E. coli* QFR include orthologs of the *E. coli* QFR, paralogs of such orthologs having fumarate reductase activity, and paralogs of the *E. coli* QFR in *E. coli* having fumarate reductase activity. The *E. coli* QFR and homologs thereof are well-recognized in the art.

The above-mentioned proteins may be functionally deleted in various combinations in a cell to enhance ethanol production in the cell and/or to enhance dual sugar (e.g., glucose and xylose) consumption. One exemplary combination comprises functional deletion of any one or more of an EI, an HPr, an FPr, and an EII^{Glc}. Another exemplary combination comprises a functional deletion of any one or more of an EI, an HPr, an FPr, and an EII^{Glc} and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of any one or more of an EI, an HPr, an FPr, and an EII^{Glc} and a functional deletion of each of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of any one or more of an EI and an EII^{Glc} and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of any one or more of an EI and an EII^{Glc} and a functional deletion of each of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of an HPr and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of an HPr and a functional deletion of each of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination

comprises a functional deletion of an FPr with a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of an FPr and a functional deletion of each of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of an HPr and an FPr and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of an HPr and an FPr and a functional deletion of each of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of any one or more of a phosphoglucose isomerase and a GntR and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of any one or more of a phosphoglucose isomerase and a GntR and a functional deletion of each of a pyruvate formate lyase and a lactate dehydrogenase. Another exemplary combination comprises a functional deletion of each of a phosphoglucose isomerase and a GntR and a functional deletion of any one or more of a pyruvate formate lyase, a lactate dehydrogenase, and a fumarate reductase. Another exemplary combination comprises a functional deletion of each of a phosphoglucose isomerase and a GntR and a functional deletion of each of a pyruvate formate lyase and a lactate dehydrogenase.

In various versions of the invention, the cell is genetically modified to comprise a recombinant gene. In most cases, the recombinant gene is configured to be expressed or overexpressed in the cell. If a cell endogenously comprises a particular gene, the gene may be modified to exchange or optimize promoters, exchange or optimize enhancers, or exchange or optimize any other genetic element to result in increased expression of the gene. Alternatively, one or more additional copies of the gene or coding sequence thereof may be introduced to the cell for enhanced expression of the gene product. If a cell does not endogenously comprise a particular gene, the gene or coding sequence thereof may be introduced to the cell for expression of the gene product. The gene or coding sequence may be incorporated into the genome of the cell or may be contained on an extrachromosomal plasmid. The gene or coding sequence may be introduced to the cell individually or may be included in an operon. Techniques for genetic manipulation are described in further detail below.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant phosphogluconate dehydratase gene. "Phosphogluconate dehydratase gene" refers to a polynucleotide that encodes or expresses a phosphogluconate dehydratase or a gene product having phosphogluconate dehydratase activity. Phosphogluconate dehydratases are also known as 6-phosphogluconate dehydratases 6-phosphogluconic dehydrases, gluconate-6-phosphate dehydratases, gluconate 6-phosphate dehydratases, 6-phosphogluconate dehydrases, and 6-phospho-D-gluconate hydro-lyases. Phosphogluconate dehydratase activity includes the activity characterized by the enzymes classified under EC 4.2.1.12. An exemplary phosphogluconate dehydratase is the Edd of *E. coli*, which is encoded by edd. An exemplary sequence of the *E. coli* Edd is SEQ ID NO:30, and an exemplary sequence of the *E. coli* edd is SEQ ID NO:29. Other phosphogluconate dehydratases include homologs of the *E. coli* Edd. Homologs of the *E. coli* Edd include orthologs of the *E. coli* Edd, paralogs of such orthologs having phosphogluconate dehydratase activity, and paralogs of the *E. coli* Edd in *E. coli* having phosphogluconate dehydratase activity. Phosphogluconate dehydratases are well-recognized in the art.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant 2-keto-4-hydroxyglutarate aldolase gene. "2-Keto-4-hydroxyglutarate aldolase gene" refers to a polynucleotide that encodes or expresses a 2-keto-4-hydroxyglutarate aldolase or a gene product having 2-keto-4-hydroxyglutarate aldolase activity. 2-Keto-4-hydroxyglutarate aldolases are also known as 4-hydroxy-2-oxoglutarate aldolases, 2-oxo-4-hydroxyglutarate aldolases, 4-hydroxy-2-oxoglutarate glyoxylate-lyases, KHG-aldolases, and KHGAs. 2-Keto-4-hydroxyglutarate aldolase activity includes the activity characterized by the enzymes classified under EC 4.1.3.16 and EC 4.1.3.42. An exemplary 2-keto-4-hydroxyglutarate aldolase is the Eda of *E. coli*, which is encoded by eda. An exemplary sequence of the *E. coli* Eda is SEQ ID NO:32, and an exemplary sequence of the *E. coli* eda is SEQ ID NO:31. Other 2-keto-4-hydroxyglutarate aldolases include homologs of the *E. coli* Eda. Homologs of the *E. coli* Eda include orthologs of the *E. coli* Eda, paralogs of such orthologs having 2-keto-4-hydroxyglutarate aldolase activity, and paralogs of the *E. coli* Eda in *E. coli* having 2-keto-4-hydroxyglutarate aldolase activity. 2-Keto-4-hydroxyglutarate aldolases are well-recognized in the art.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant 2-keto-3-deoxy-6-phosphogluconate aldolase gene. "2-Keto-3-deoxy-6-phosphogluconate aldolase gene" refers to a polynucleotide that encodes or expresses a 2-keto-3-deoxy-6-phosphogluconate aldolase or a gene product having 2-keto-3-deoxy-6-phosphogluconate aldolase activity. 2-Keto-3-deoxy-6-phosphogluconate aldolases are also known as 2-dehydro-3-deoxy-phosphogluconate aldolases, 2-dehydro-3-deoxy-D-gluconate-6-phosphate D-glyceraldehyde-3-phosphate-lyases, KDPG-aldolases, phospho-2-dehydro-3-deoxygluconate aldolases, and phospho-2-keto-3-deoxygluconate aldolases. 2-Keto-3-deoxy-6-phosphogluconate aldolase activity includes the activity characterized by the enzymes classified under EC 4.1.2.14 and EC 4.1.2.55. An exemplary 2-keto-3-deoxy-6-phosphogluconate aldolase is the Eda of *E. coli*, which is encoded by eda. An exemplary sequence of the *E. coli* Eda is SEQ ID NO:32, and an exemplary sequence of the *E. coli* eda is SEQ ID NO:31. Other 2-keto-3-deoxy-6-phosphogluconate aldolases include homologs of the *E. coli* Eda. Homologs of the *E. coli* Eda include orthologs of the *E. coli* Eda, paralogs of such orthologs having 2-keto-3-deoxy-6-phosphogluconate aldolase activity, and paralogs of the *E. coli* Eda in *E. coli* having 2-keto-3-deoxy-6-phosphogluconate aldolase activity. 2-Keto-3-deoxy-6-phosphogluconate aldolases are well-recognized in the art.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant oxaloacetate decarboxylase gene. "Oxaloacetate decarboxylase gene" refers to a polynucleotide that encodes or expresses an oxaloacetate decarboxylase or a gene product having oxaloacetate decarboxylase activity. Oxaloacetate decarboxylases are also known as oxaloacetate β-decarboxylases and oxaloacetate carboxy-lyases. Oxaloacetate decarboxylase activity includes the activity characterized by the enzymes classified under EC 4.1.1.3 and EC 1.1.1.38. An exemplary oxaloacetate decarboxylase is the Eda of *E. coli*, which is encoded by eda. An exemplary sequence of the *E. coli* Eda is SEQ ID

NO:32, and an exemplary sequence of the *E. coli* eda is SEQ ID NO:31. Other oxaloacetate decarboxylases include homologs of the *E. coli* Eda. Homologs of the *E. coli* Eda include orthologs of the *E. coli* Eda, paralogs of such orthologs having oxaloacetate decarboxylase activity, and paralogs of the *E. coli* Eda in *E. coli* having oxaloacetate decarboxylase activity. Oxaloacetate decarboxylases are well-recognized in the art.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant pyruvate decarboxylase gene. "Pyruvate decarboxylase gene" refers to a polynucleotide that encodes or expresses a pyruvate decarboxylase or a gene product having pyruvate decarboxylase activity. Pyruvate decarboxylase activity includes the activity characterized by the enzymes classified under EC 4.1.1.1. An exemplary pyruvate decarboxylase is the PDC of *Zymomonas mobilis*, which is encoded by pdc. An exemplary sequence of the *Z. mobilis* PDC is SEQ ID NO:34, and an exemplary sequence of the *Z. mobilis* pdc is SEQ ID NO:33. Other pyruvate decarboxylases include homologs of the *Z. mobilis* PDC. Homologs of the *Z. mobilis* PDC include orthologs of the *Z. mobilis* PDC, paralogs of such orthologs having pyruvate decarboxylase activity, and paralogs of the *Z. mobilis* PDC in *E. coli* having pyruvate decarboxylase activity. Pyruvate decarboxylases are well-recognized in the art.

In some versions of the invention, the cells are genetically modified to express or overexpress a recombinant alcohol dehydrogenase gene. "Alcohol dehydrogenase gene" refers to a polynucleotide that encodes or expresses an alcohol dehydrogenase or a gene product having alcohol dehydrogenase activity. Alcohol dehydrogenase activity includes the activity characterized by the enzymes classified under EC 1.1.1.1. An exemplary alcohol dehydrogenase is the ADH2 of *Zymomonas mobilis*, which is encoded by adhB. An exemplary sequence of the *Z. mobilis* ADH2 is SEQ ID NO:36, and an exemplary sequence of the *Z. mobilis* adhB is SEQ ID NO:35. Other alcohol dehydrogenases include homologs of the *Z. mobilis* ADH2. Homologs of the *Z. mobilis* ADH2 include orthologs of the *Z. mobilis ADH2*, paralogs of such orthologs having alcohol dehydrogenase activity, and paralogs of the *Z. mobilis* ADH2 in *Z. mobilis* having alcohol dehydrogenase activity. Alcohol dehydrogenases are well-recognized in the art.

The recombinant pyruvate decarboxylase gene and/or the recombinant alcohol dehydrogenase gene can be included in a microorganism comprising a functional deletion of any of the genes or gene products, or combinations thereof, described herein.

The cells of the invention may be genetically altered to functionally delete, express, or overexpress homologs of any of the specific genes or gene products explicitly described herein. Proteins and/or protein sequences are "homologous" when they are derived, naturally or artificially, from a common ancestral protein or protein sequence. Similarly, nucleic acids and/or nucleic acid sequences are homologous when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Nucleic acid or gene product (amino acid) sequences of any known gene, including the genes or gene products described herein, can be determined by searching any sequence databases known the art using the gene name or accession number as a search term. Common sequence databases include GenBank (http://www.ncbi.nlm.nih.gov/genbank/), ExPASy (http://expasy.org/), KEGG (www.genome.jp/kegg/), among others. Homology is generally inferred from sequence similarity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of similarity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence similarity (e.g., identity) over 50, 100, 150 or more residues (nucleotides or amino acids) is routinely used to establish homology (e.g., over the full length of the two sequences to be compared). Higher levels of sequence similarity (e.g., identity), e.g., 30%, 35% 40%, 45% 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% or more, can also be used to establish homology. Accordingly, homologs of the genes or gene products described herein include genes or gene products having at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to the genes or gene products described herein. Methods for determining sequence similarity percentages (e.g., BLASTP and BLASTN using default parameters) are described herein and are generally available. The homologous proteins should demonstrate comparable activities and, if an enzyme, participate in the same or analogous pathways. Homologs include orthologs and paralogs. "Orthologs" are genes and products thereof in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same or similar function in the course of evolution. Paralogs are genes and products thereof related by duplication within a genome. As used herein, "orthologs" and "paralogs" are included in the term "homologs."

For sequence comparison and homology determination, one sequence typically acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence based on the designated program parameters. A typical reference sequence of the invention is a nucleic acid or amino acid sequence corresponding to the genes or gene products described herein.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see Current Protocols in Molecular Biology, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2008)).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity for purposes of defining homologs is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer

HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always>0) and N (penalty score for mismatching residues; always<0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001. The above-described techniques are useful in identifying homologous sequences for use in the methods described herein.

The terms "identical" or "percent identity", in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described above (or other algorithms available to persons of skill) or by visual inspection.

The phrase "substantially identical" in the context of two nucleic acids or polypeptides refers to two or more sequences or subsequences that have at least about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90, about 95%, about 98%, or about 99% or more nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. Such "substantially identical" sequences are typically considered to be "homologous", without reference to actual ancestry. Preferably, the "substantial identity" exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably, the sequences are substantially identical over at least about 150 residues, at least about 250 residues, or over the full length of the two sequences to be compared.

Terms used herein pertaining to genetic manipulation are defined as follows.

Deletion: The removal of one or more nucleotides from a nucleic acid molecule or one or more amino acids from a protein, the regions on either side being joined together.

Derived: When used with reference to a nucleic acid or protein, "derived" means that the nucleic acid or polypeptide is isolated from a described source or is at least 70%, 80%, 90%, 95%, 99%, or more identical to a nucleic acid or polypeptide included in the described source.

Endogenous: As used herein with reference to a nucleic acid molecule and a particular cell, "endogenous" refers to a nucleic acid sequence or polypeptide that is in the cell and was not introduced into the cell using recombinant engineering techniques. For example, an endogenous gene is a gene that was present in a cell when the cell was originally isolated from nature.

Exogenous: As used herein with reference to a nucleic acid molecule or polypeptide in a particular cell, "exogenous" refers to any nucleic acid molecule or polypeptide that does not originate from that particular cell as found in nature. Thus, a non-naturally-occurring nucleic acid molecule or protein is considered to be exogenous to a cell once introduced into the cell. A nucleic acid molecule or protein that is naturally-occurring also can be exogenous to a particular cell. For example, an entire coding sequence isolated from cell X is an exogenous nucleic acid with respect to cell Y once that coding sequence is introduced into cell Y, even if X and Y are the same cell type. The term "heterologous" is used herein interchangeably with "exogenous."

Expression: The process by which a gene's coded information is converted into the structures and functions of a cell, such as a protein, transfer RNA, or ribosomal RNA. Expressed genes include those that are transcribed into mRNA and then translated into protein and those that are transcribed into RNA but not translated into protein (for example, transfer and ribosomal RNAs).

Introduce: When used with reference to genetic material, such as a nucleic acid, and a cell, "introduce" refers to the delivery of the genetic material to the cell in a manner such that the genetic material is capable of being expressed within the cell. Introduction of genetic material includes both transformation and transfection. Transformation encompasses techniques by which a nucleic acid molecule can be introduced into cells such as prokaryotic cells or non-animal eukaryotic cells. Transfection encompasses techniques by which a nucleic acid molecule can be introduced into cells such as animal cells. These techniques include but are not limited to introduction of a nucleic acid via conjugation, electroporation, lipofection, infection, and particle gun acceleration.

Isolated: An "isolated" biological component (such as a nucleic acid molecule, polypeptide, or cell) has been substantially separated or purified away from other biological components in which the component naturally occurs, such as other chromosomal and extrachromosomal DNA and RNA and proteins. Nucleic acid molecules and polypeptides that have been "isolated" include nucleic acid molecules and polypeptides purified by standard purification methods. The term also includes nucleic acid molecules and polypeptides prepared by recombinant expression in a cell as well as chemically synthesized nucleic acid molecules and polypeptides. In one example, "isolated" refers to a naturally-occurring nucleic acid molecule that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived.

Nucleic acid: Encompasses both RNA and DNA molecules including, without limitation, cDNA, genomic DNA, and mRNA. Nucleic acids also include synthetic nucleic acid molecules, such as those that are chemically synthesized or recombinantly produced. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid molecule can be the sense strand, the antisense strand, or both. In addition, the nucleic acid can be circular or linear.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. An origin of replication is operably linked to a coding sequence if the origin of replication controls the replication or copy number of the nucleic acid in the cell. Operably linked nucleic acids may or may not be contiguous.

Operon: Configurations of separate genes that are transcribed in tandem as a single messenger RNA are denoted as operons. Thus, a set of in-frame genes in close proximity under the transcriptional regulation of a single promoter constitutes an operon. Operons may be synthetically generated using the methods described herein.

Overexpress: When a gene is caused to be transcribed at an elevated rate compared to the endogenous or basal transcription rate for that gene. In some examples, overexpression additionally includes an elevated rate of translation of the gene compared to the endogenous translation rate for that gene. Methods of testing for overexpression are well known in the art, for example transcribed RNA levels can be assessed using RT-PCR and protein levels can be assessed using SDS page gel analysis.

Recombinant: A recombinant nucleic acid molecule or polypeptide is one that has a sequence that is not naturally occurring, has a sequence that is made by an artificial combination of two otherwise separated segments of sequence, or both. This artificial combination can be achieved, for example, by chemical synthesis or by the artificial manipulation of isolated segments of nucleic acid molecules or polypeptides, such as genetic engineering techniques. "Recombinant" is also used to describe nucleic acid molecules that have been artificially manipulated but contain the same regulatory sequences and coding regions that are found in the organism from which the nucleic acid was isolated, such as an introduced additional copy of a nucleic acid molecule naturally present in the organism. A recombinant cell or microorganism is one that contains an exogenous nucleic acid molecule, such as a recombinant nucleic acid molecule.

Recombinant cell: A cell that comprises a recombinant nucleic acid.

Vector or expression vector: An entity comprising a nucleic acid molecule that is capable of introducing the nucleic acid, or being introduced with the nucleic acid, into a cell for expression of the nucleic acid. A vector can include nucleic acid sequences that permit it to replicate in the cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Examples of suitable vectors are found below.

Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below.

Exogenous nucleic acids can be introduced stably or transiently into a cell using techniques well known in the art, including electroporation, calcium phosphate precipitation, DEAE-dextran mediated transfection, liposome-mediated transfection, conjugation, transduction, and the like. For stable transformation, a nucleic acid can further include a selectable marker. Suitable selectable markers include antibiotic resistance genes that confer, for example, resistance to neomycin, tetracycline, chloramphenicol, or kanamycin, genes that complement auxotrophic deficiencies, and the like. (See below for more detail.)

Various embodiments of the invention use an expression vector that includes a heterologous nucleic acid encoding a protein involved in a metabolic or biosynthetic pathway. Suitable expression vectors include, but are not limited to viral vectors, phage vectors, bacteriophage vectors, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, Pl-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for cells of interest.

Useful vectors can include one or more selectable marker genes to provide a phenotypic trait for selection of transformed cells. The selectable marker gene encodes a protein necessary for the survival or growth of transformed cells grown in a selective culture medium. Cells not transformed with the vector containing the selectable marker gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. In alternative embodiments, the selectable marker gene is one that encodes dihydrofolate reductase or confers neomycin resistance (for use in eukaryotic cell culture), or one that confers tetracycline or ampicillin resistance (for use in a prokaryotic cell, such as *E. coli*).

The coding sequence in the expression vector is operably linked to an appropriate expression control sequence (promoters, enhancers, and the like) to direct synthesis of the encoded gene product. Such promoters can be derived from microbial or viral sources. Depending on the cell/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. can be used in the expression vector (see e.g., Bitter et al. (1987) *Methods in Enzymology,* 153:516-544).

Suitable promoters for use in prokaryotic cells include but are not limited to: promoters capable of recognizing the T4, T3, Sp6, and T7 polymerases; the P_R and P_L promoters of bacteriophage lambda; the trp, recA, heat shock, and lacZ promoters of *E. coli*; the alpha-amylase and the sigma-specific promoters of *B. subtilis*; the promoters of the bacteriophages of *Bacillus; Streptomyces* promoters; the int promoter of bacteriophage lambda; the bla promoter of the beta-lactamase gene of pBR322; and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters are reviewed by Glick, *J. Ind. Microbiol.* 1:277 (1987); Watson et al, Molecular Biology of the Gene, 4th Ed., Benjamin Cummins (1987); and Sambrook et al., In: *Molecular Cloning: A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press (2001).

Non-limiting examples of suitable promoters for use within a eukaryotic cell are typically viral in origin and include the promoter of the mouse metallothionein I gene (Hamer et al. (1982) *J. Mol. Appl. Gen.* 1:273); the TK promoter of Herpes virus (McKnight (1982) *Cell* 31:355); the SV40 early promoter (Benoist et al. (1981) *Nature* (London) 290:304); the Rous sarcoma virus promoter; the cytomegalovirus promoter (Foecking et al. (1980) *Gene* 45:101); and the yeast gal4 gene promoter (Johnston et al. (1982) *PNAS* (USA) 79:6971; Silver et al. (1984) *PNAS* (USA) 81:5951.

Coding sequences can be operably linked to an inducible promoter. Inducible promoters are those wherein addition of an effector induces expression. Suitable effectors include proteins, metabolites, chemicals, or culture conditions capable of inducing expression. Suitable inducible promoters include but are not limited to the lac promoter (regulated by IPTG or analogs thereof), the lacUV5 promoter (regulated by IPTG or analogs thereof), the tac promoter (regulated by IPTG or analogs thereof), the trc promoter (regulated by IPTG or analogs thereof), the araBAD promoter (regulated by L-arabinose), the phoA promoter (regulated by phosphate starvation), the recA promoter (regulated by nalidixic acid), the proU promoter (regulated by osmolarity changes), the cst-1 promoter (regulated by glucose starvation), the tetA promoter (regulated by tetracycline), the cadA promoter (regulated by pH), the nar promoter (regulated by anaerobic conditions), the p_L promoter (regulated by thermal shift), the cspA promoter (regulated by thermal shift), the T7 promoter (regulated by thermal shift), the T7-lac promoter (regulated by IPTG), the T3-lac promoter (regulated by IPTG), the T5-lac promoter (regulated by IPTG), the T4 gene 32 promoter (regulated by T4 infection), the nprM-lac promoter (regulated by IPTG), the VHb promoter (regulated by oxygen), the metallothionein promoter (regulated by heavy metals), the MMTV promoter (regulated by steroids such as dexamethasone) and variants thereof.

Alternatively, a coding sequence can be operably linked to a repressible promoter. Repressible promoters are those wherein addition of an effector represses expression. Examples of repressible promoters include but are not limited to the trp promoter (regulated by tryptophan); tetracycline-repressible promoters, such as those employed in the "TET-OFF"-brand system (Clontech, Mountain View, Calif.); and variants thereof.

In some versions, the cell is genetically modified with a heterologous nucleic acid encoding a biosynthetic pathway gene product that is operably linked to a constitutive promoter. Suitable constitutive promoters are known in the art.

In some versions, the cell is genetically modified with an exogenous nucleic acid encoding a single protein. In other embodiments, a modified cell is one that is genetically modified with exogenous nucleic acids encoding two or more proteins. Where the cell is genetically modified to express two or more proteins, those nucleic acids can each be contained in a single or in separate expression vectors. When the nucleic acids are contained in a single expression vector, the nucleotide sequences may be operably linked to a common control element (e.g., a promoter), that is, the common control element controls expression of all of the coding sequences in the single expression vector.

When the cell is genetically modified with heterologous nucleic acids encoding two or more proteins, one of the nucleic acids can be operably linked to an inducible promoter, and one or more of the nucleic acids can be operably linked to a constitutive promoter. Alternatively, all can be operably linked to inducible promoters or all can be operably linked to constitutive promoters.

Nucleic acids encoding enzymes desired to be expressed in a cell may be codon-optimized for that particular type of cell. Codon optimization can be performed for any nucleic acid by "OPTIMUMGENE"-brand gene design system by GenScript (Piscataway, N.J.).

The introduction of a vector into a bacterial cell may be performed by protoplast transformation (Chang and Cohen (1979) *Molecular General Genetics,* 168:111-115), using competent cells (Young and Spizizen (1961) *Journal of Bacteriology,* 81:823-829; Dubnau and Davidoff-Abelson (1971) *Journal of Molecular Biology,* 56: 209-221), electroporation (Shigekawa and Dower (1988) *Biotechniques,* 6:742-751), or conjugation (Koehler and Thorne (1987) *Journal of Bacteriology,* 169:5771-5278). Commercially available vectors for expressing heterologous proteins in bacterial cells include but are not limited to pZERO, pTrc99A, pUC19, pUC18, pKK223-3, pEX1, pCAL, pET, pSPUTK, pTrxFus, pFastBac, pThioHis, pTrcHis, pTrcHis2, and pLEx, in addition to those described in the following Examples.

Methods for transforming yeast cells with heterologous DNA and producing heterologous polypeptides therefrom are disclosed by Clontech Laboratories, Inc., Palo Alto, Calif., USA (in the product protocol for the "YEASTMAKER"-brand yeast transformation system kit); Reeves et al. (1992) *FEMS Microbiology Letters* 99:193-198; Manivasakam and Schiestl (1993) *Nucleic Acids Research* 21(18): 4414-5; and Ganeva et al. (1994) *FEMS Microbiology Letters* 121:159-64. Expression and transformation vectors for transformation into many yeast strains are available. For example, expression vectors have been developed for the following yeasts: *Candida albicans* (Kurtz, et al. (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze et al. (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson et al. (1986) *J. Gen. Microbiol.* 132:3459) and Roggenkamp et al. (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das et al. (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt et al. (1983) *J. Bacteriol.* 154:737) and Van den Berg et al. (1990) Bio/Technology 8:135); *Pichia quillerimondii* (Kunze et al. (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg et al. (1985) *Mol. Cell. Biol.* 5:3376; U.S. Pat. Nos. 4,837, 148; and 4,929,555); *Saccharomyces cerevisiae* (Hinnen et al. (1978) *Proc. Natl. Acad. Sci.* USA 75:1929 and Ito et al. (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach et al. (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow et al. (1985) *Curr. Genet.* 10:380-471 and Gaillardin et al. (1985) *Curr. Genet.* 10:49).

Suitable procedures for transformation of *Aspergillus* cells are described in EP 238 023 and U.S. Pat. No. 5,679, 543. Suitable methods for transforming *Fusarium* species are described by Malardier et al., *Gene,* 1989, 78:147-56 and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J. N. and Simon, M. I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito et al. (1983) *Journal of Bacteriology,* 153: 163; and Hinnen et al. (1978) *PNAS* USA, 75:1920.

The microorganisms of the invention are particularly suited to co-consuming glucose with non-glucose carbohydrates, such as xylose. Accordingly, methods of the invention comprise culturing a microorganism of the invention in a medium comprising glucose and xylose. The xylose may be present at any level within the medium. The glucose and

xylose may each independently be present in the medium at least at 5 g/l, 10 g/l, 15 g/l, 20 g/l, 25 g/l, 30 g/l, 35 g/l, 40 g/l, 45 g/l, 50 g/l, 55 g/l, 60 g/l, 65 g/l, 70 g/l, 75 g/l, 80 g/l, 85 g/l, 90 g/l, 95 g/l, 100 g/l, 110 g/l, 120 g/l, 130 g/l or even more. The medium may also comprise other components, including those derived from a biomass or lignocellulosic material such as cellobiose, arabinose, and rhamnose.

In culturing the microorganism, the microorganism may consume at least about 1%, 2.5%, 5%, 7.5%, or 10% of the initial amount of xylose in the medium during the time the microorganism consumes about 10% of the initial amount of glucose in the medium; at least about 5%, 10%, 15%, or 20% of the initial amount of xylose in the medium during the time the microorganism consumes about 20% of the initial amount of glucose in the medium; at least about 10%, 15%, 20%, 25%, or 30% of the initial amount of xylose in the medium during the time the microorganism consumes about 30% of the initial amount of glucose in the medium; at least about 10%, 20%, 25%, 30%, 35%, or 40% of the initial amount of xylose in the medium during the time the microorganism consumes about 40% of the initial amount of glucose in the medium; at least about 10%, 20%, 30%, 35%, 40%, 45%, or 50% of the initial amount of xylose in the medium during the time the microorganism consumes about 50% of the initial amount of glucose in the medium; at least about 20%, 40%, 45%, 50%, 55%, or 60% of the initial amount of xylose in the medium during the time the microorganism consumes about 60% of the initial amount of glucose in the medium; at least about 40%, 50%, 55%, 60%, 65%, or 70% of the initial amount of xylose in the medium during the time the microorganism consumes about 70% of the initial amount of glucose in the medium; at least about 50%, 60%, 65%, 70%, 75%, or 80% of the initial amount of xylose in the medium during the time the microorganism consumes about 80% of the initial amount of glucose in the medium; or at least about 50%, 60%, 70%, 75%, 80%, 85%, or 90% of the initial amount of xylose in the medium during the time the microorganism consumes about 90% of the initial amount of glucose in the medium.

In some versions, the medium comprises a biomass hydrolysate. Biomass is biological material derived from living or once-living organisms. Biomass can be from plant, animal, or other organic material. Biomass is carbon based and is composed of a mixture of organic molecules containing hydrogen, usually including atoms of oxygen, often nitrogen and also small quantities of other atoms, including alkali, alkaline earth and heavy metals. The biomass hydrolysate for use in the present invention can be produced from any biomass feedstock. Exemplary types of biomass feedstocks include sucrose-rich feedstocks such as sugar cane; starchy materials, such as corn grain; and lignocellulosic biomass, such as coastal Bermuda grass, switchgrass (*Pancium virgatum*), corn cobs, corn stover, cotton seed hairs, grasses, hardwood, hardwood stems, maple, leaves, newspaper, sugarcane bagasse, nut shells, paper, primary wastewater solids, softwood, softwood stems, pine loblolly pine, solid cattle manure, sorted refuse, grain hulls, swine waste, switchgrass, waste papers from chemical pulps, wheat straw, wood, wood chips, wood pulp, woody residues *Miscanthus*, date palm (*Phoenix dectylifera*), oil palm, Sorghum, and *Arundo donax*.

Prior to hydrolysis, the biomass feedstock may be pretreated or non-pretreated. Pretreatment of biomass feedstock removes a large proportion of the lignin and other materials and enhances the porosity of the biomass prior to hydrolysis. The biomass feedstock may be pretreated by any method. Exemplary pretreatments include chipping, grinding, milling, steam pretreatment, ammonia fiber expansion (AFEX, also referred to as ammonia fiber explosion), ammonia recycle percolation (ARP), CO_2 explosion, steam explosion, ozonolysis, wet oxidation, acid hydrolysis, dilute-acid hydrolysis, alkaline hydrolysis, ionic liquid, organosolv, and pulsed electrical field treatment, among others. See, e.g., Kumar et al. 2009 and da Costa Lopes et al. 2013.

The pretreated or non-pretreated biomass may be hydrolyzed by any suitable method. Hydrolysis converts biomass polymers to fermentable sugars, such as glucose and xylose, and other monomeric or oligomeric components. Exemplary hydrolysis methods include enzymatic hydrolysis (e.g., with cellulases or other enzymes), acid hydrolysis (e.g., with sulfurous, sulfuric, hydrochloric, hydrofluoric, phosphoric, nitric, and/or formic acids), and ionic liquid hydrolysis (e.g., with 1-ethyl-3-methylimidazolium chloride) (Binder et al. 2010) among other methods. The hydrolysate may be in aqueous solution, concentrated, or dehydrated.

The microorganisms of the invention are particularly suited for producing ethanol from the consumption of carbohydrates such as glucose and/or xylose. Accordingly, methods of the invention comprise culturing a microorganism in a medium for a time sufficient to produce an amount of ethanol. The medium in such culturing may comprise glucose and xylose and/or may be a biomass hydrolysate as described above. The culturing may produce ethanol in an amount of at least about 10 mM, 25 mM, 50 mM, 75 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 550 mM, 600 mM, 650 mM, 700 mM, 750 mM, 800 mM, 850 mM, 900 mM or more. The culturing may produce a yield of ethanol based on the consumption of glucose and xylose of at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99% or more.

Prior to the culturing described herein, the microorganisms of the invention may be adapted to growth in the presence of certain components through adaptive evolution. Such components may comprise any one or more of glucose, xylose, ethanol, and biomass hydrolysate. The adapting may comprise serially passing the microorganisms in media comprising constant or increasing amounts of one or more of the components. In some versions, the adapting may comprise serially passing the microorganisms in media comprising constant or increasing amounts of one or more of the components to the exclusion of another one or more of the components. In some versions, the adapting may comprise serially passing the microorganisms in media comprising constant or increasing amounts of a first set of components and then serially passing the microorganisms in media comprising constant or increasing amounts of a second set of components, wherein the first set of components is different from the second set of components. In some versions, the adapting may comprise serially passing the microorganisms in media comprising constant or increasing amounts of a first set of components, then serially passing the microorganisms in media comprising constant or increasing amounts of a second set of components, and then serially passing the microorganisms in media comprising constant or increasing amounts of a third set of components, wherein at least two or all three of the first set of components, the second set of components and the third set of components are different from each other.

The elements and method steps described herein can be used in any combination whether explicitly described or not.

All combinations of method steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

As used herein, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise.

Numerical ranges as used herein are intended to include every number and subset of numbers contained within that range, whether specifically disclosed or not. Further, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 2 to 8, from 3 to 7, from 5 to 6, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

All patents, patent publications, and peer-reviewed publications (i.e., "references") cited herein are expressly incorporated by reference to the same extent as if each individual reference were specifically and individually indicated as being incorporated by reference. In case of conflict between the present disclosure and the incorporated references, the present disclosure controls.

It is understood that the invention is not confined to the particular construction and arrangement of parts herein illustrated and described, but embraces such modified forms thereof as come within the scope of the claims.

EXAMPLE

Introduction

Efficient conversion of lignocellulose-derived sugars to ethanol and other biofuels is a crucial step in sustainable bioenergy production from biomass. Glucose and xylose are the major sugars in pretreated lignocellulosic hydrolysate, and can both be converted by microorganisms into ethanol and other biofuels. However, microbial conversion of these sugars in lignocellulosic hydrolysate is hindered by the fact that microbes preferentially consume glucose first and do not consume xylose after glucose is depleted. This has been attributed to stresses associated with growth in hydrolysate (e.g., inhibitors produced from pretreatment, ethanol produced by fermentation, and high osmolarity).

Computational models of metabolic networks have been successfully used to study and engineer microbial metabolism to produce valuable chemicals. We used genome-scale metabolic models of *Escherichia coli* to identify gene knockout strategies to improve co-utilization of glucose and xylose in lignocellulosic hydrolysate (FIG. **1**). Using the computational predictions, we constructed gene knockout mutants with inserted *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase genes to increase conversion of sugars to ethanol.

The constructed *E. coli* strains co-utilized glucose and xylose anaerobically in minimal media, but their growth and glucose uptake rates were much slower than the parental *E. coli* strain's. The engineered strains were then adaptively evolved in minimal media containing (1) glucose, (2) glucose and xylose, and (3) glucose and gradually increasing concentrations of ethanol. The evolved strains were able to simultaneously convert glucose and xylose into ethanol when grown in a synthetic hydrolysate (SynH) medium. In addition, two of the evolved strains co-utilized most of the glucose and xylose in ammonia fiber explosion (AFEX) pre-treated corn stover and switchgrass hydrolysates. The developed strains show significantly improved conversion of sugars into ethanol in lignocellulosic hydrolysates and provide a platform that may be further engineered to produce next-generation biofuels.

Material and Methods

Computational Strain Design for Improved Conversion of Glucose and Xylose to Ethanol

Genome-scale metabolic and transcriptional regulatory models of *Escherichia coli* (Reed et al. 2003, Covert et al. 2004, Feist et al. 2007, Barua et al. 2010, Orth et al. 2011) were used to identify genetic perturbations to improve the conversion of glucose and xylose to ethanol. The *E. coli* metabolic models were augmented with reactions catalyzed by *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase. A bi-level strain design method, OptORF (Kim et al. 2010), was first used to find metabolic gene or transcription factor deletions that would couple cellular growth and ethanol production in a minimal medium containing glucose and xylose. An uptake rate of 10 mmol/gDW/hr was used for glucose or xylose to simulate minimal media containing either glucose or xylose, and glucose uptake rate of 6 mmol/gDW/hr and xylose uptake rate of 4 mmol/gDW/hr were used to simulate minimal media containing both glucose and xylose. A flux prediction method, RELATCH (Kim et al. 2012), was used to find metabolic gene deletions that would improve xylose utilization in the presence of glucose. Among the genetic perturbation strategies identified by OptORF, the strategies that include the gene deletions identified by RELATCH were selected for experimental characterization.

Strain Construction and Adaptive Evolution

E. coli K-12 MG1655 was used to construct the strains based on the computational design. A PET cassette containing *Z. mobilis* pyruvate decarboxylase (pdc_{Zm}) and alcohol dehydrogenase ($adhB_{Zm}$), and chloramphenicol resistance marker (cat) was inserted into the NW locus as described in a previous study (Schwalbach et al. 2012). The rest of genes were subsequently deleted using P1 transduction or other previously described methods (Baba et al. 2006, Datsenko et al. 2000).

TABLE 1

List of plasmids and strains

Plasmids/ Strains	Genotype
pPET	pdc_{Zmo} $adhB_{Zmo}$ (pJGG2, Gardner et al. 2010)
Parent	*E. coli* K-12 MG1655
JK10	ldhA::kan pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat)
JK10 pPET	JK10 with pPET
JK20	ldhA::kan pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) Δpgi ΔgntR
JK20 pPET	JK20 with pPET
JK20E	JK20 adaptively evolved in M9 + glucose, xylose, ethanol
JK20E pPET	Isolate of JK20E with pPET
JK30	ldhA::kan pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) ΔptsH
JK30E	JK30 adaptively evolved in M9 + xylose
JK31	ΔldhA pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) ΔptsH fruB::kan (glc^-) (from JK30)
JK32	ΔldhA pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) ΔptsH fruB::kan (glc^+) (isolate of JK31 grown on M9 glucose agar plates)
JK32 pPET	JK32 with pPET
JK32E	JK32 adaptively evolved in M9 + glucose, xylose, ethanol
JK32E pPET	isolate of JK32E with pPET
JK33E	ΔldhA pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) ΔptsH ΔfruB frdA::kan (derived from isolate from JK32E)
JK33E pPET	JK33E with pPET

The constructed strain JK30 was adaptively evolved at 37° C. by transferring cells to a fresh M9 minimal medium containing 2 g/L xylose at mid-exponential phase repeatedly. The adaptively evolved strain after 7 serial transfers was designated as JK30E. The strain JK31, which was derived from JK30, initially did not grow on glucose as a sole carbon source. Aliquots of cells were plated on glucose M9 agar

plates and colonies were found after incubating at 37° C. for a few days, and an isolate was found that was able to grow in a M9 medium containing 2 g/L glucose (designated as JK32). The strains JK20 and JK32 were then each independently adaptively evolved at 37° C. by transferring cells to a fresh medium at mid-exponential phase, repeatedly. First, cells were transferred five times in M9 minimal media containing 10 g/L glucose and five times in M9 minimal media containing 6 g/L glucose and 4 g/L xylose to improve sugar uptake. Next, cells were transferred five times in M9 minimal media containing 10 g/L glucose with ethanol concentration from 1% to 5% (v/v) increased by 1% at each transfer. Cells were then transferred five times in M9 minimal media containing 10 g/L glucose and 5% ethanol to increase ethanol tolerance. Overall, the strains JK20 and JK32 were adaptively evolved independently for a total of ~100 generations, and the evolved strains were designated as JK20E and JK32E, respectively. After the adaptive evolution, an additional copy of the PET cassette with gentamycin resistance marker on a plasmid (pPET) (Schwalbach et al. 2012) was transformed into the strains and single colonies were isolated on LB agar plates containing gentamycin. Strains containing the pPET plasmids are labeled accordingly (e.g., JK20E pPET).

Strain Characterization and Growth Condition

The initial characterization of the constructed strains was performed anaerobically in a M9 medium containing 6 g/L glucose and 4 g/L xylose to evaluate sugar utilization and ethanol production. The medium was flushed with N_2 gas and cultures were maintained anaerobic in Hungate tubes. Subsequent characterization in a synthetic hydrolysate medium (without lignocellulose-derived inhibitors) containing 60 g/L glucose and 30 g/L xylose (Keating et al. 2014) was done using 125 ml flasks with 50 ml of working volume in an anaerobic chamber sparged with 80% N_2, 10% CO_2, and 10% H_2 gas. In order to prevent the pH of cultures from decreasing significantly due to succinate production, 300 mM of phosphate buffer at pH 7 was added to the synthetic hydrolysate medium in flask experiments.

Fermentation experiments were conducted in duplicates using 250 ml mini-bioreactors (Applikon Biotechnology) with 100 ml of synthetic hydrolysate (SynH), ammonia fiber explosion (AFEX)-treated corn stover hydrolysate (ACSH), or AFEX-treated switchgrass hydrolysate (ASGH). The bioreactors were sparged with 95% N_2 and 5% CO_2 mixture gas at a flow rate of 20 ml/min, stirred at 500 rpm, and maintained at pH 7 and 37° C. Due to the sparging, the ethanol was evaporated from the culture and the amount of evaporated ethanol was estimated by a simple mass transfer model using data from an independent ethanol evaporation experiment.

Results

Computational Strain Designs to Improve Conversion of Glucose and Xylose to Ethanol

We identified genetic perturbations that would improve conversion of glucose and xylose to ethanol using genome-scale metabolic and transcriptional regulatory models of *E. coli*. The lactate dehydrogenase and pyruvate formate-lyase reactions were removed from the *E. coli* metabolic models and the *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase reactions were added to represent the ΔldhA pflB::(pdc_{Zmo} $adhB_{Zmo}$ cat) genotype of a base strain (JK10) used in this study.

First, we used OptORF to find metabolic or transcriptional regulatory gene deletions that would improve ethanol production at the maximal growth rate. Metabolic models (Reed et al. 2003, Feist et al. 2007) or integrated metabolic and transcriptional regulatory models (Covert et al. 2004, Barua et al. 2010) were employed to generate diverse sets of genetic perturbation strategies to couple growth and ethanol production from glucose and/or xylose.

We then used RELATCH to find metabolic gene deletions that would improve the xylose uptake in a minimal medium containing glucose and xylose. The phenotype and gene expression of GLBRCE1 *E. coli* strain (Keating et al. 2014) was used to generate a reference metabolic state for RELATCH predictions. We evaluated the effect of single gene deletions on the xylose uptake rate under two different cases where cells are using only glucose or both glucose and xylose in the reference state. Among the identified gene deletions that would improve the xylose uptake rate in both RELATCH simulation cases, the deletion of phosphoglucoisomerase (pgi) or phosphoenolpyruvate phosphotransferase system (ptsH or ptsI) was also part of the strategies identified by OptORF. In the strategies involving the pgi deletion, the deletion of GntR transcriptional repressor was also found to remove the repression of Entner-Doudoroff pathway genes. We constructed two *E. coli* strains based on these results (Table 2).

TABLE 2

Computationally predicted growth and ethanol production for different strains

Strains	Rate (mmol/gDW/hr) Glucose Uptake	Xylose Uptake	Ethanol Production	Growth Rate (1/hr)	Ethanol Yield (%)
JK10 and JK10 pPET	10	5	22.6	0.37	79.7
JK20 and JK20 pPET	10	5	23.8	0.29	84.1
JK30 and JK30 pPET	10	5	23.0	0.34	81.2

Co-Utilization of Glucose and Xylose by Engineered *E. coli* Strains in a Minimal Medium

The two computationally designed strains JK20 and JK30 were grown anaerobically in a M9 minimal medium containing 6 g/L of glucose and 4 g/L of xylose. Both JK20 and JK30 co-utilized glucose and xylose and produced ethanol (FIGS. **2**A and **2**B). While JK20 produced mainly ethanol from glucose and xylose, JK30 also produced some succinate and acetate as co-products. Since the overall conversion was slow in both strains, they were adaptively evolved in a M9 minimum medium containing 2 g/L xylose to improve the conversion of xylose to ethanol. However, after 7 serial transfers, we found that evolved strain JK30E showed a 2-fold increase in glucose uptake but had a 2-fold decrease in xylose uptake (FIG. **2**C) when grown in M9 media with glucose and xylose. This was unexpected since the cells were adaptively evolved in the absence of glucose.

Sequencing of JK30E found mutations in the fruR gene, exclusively either a point mutation (N50K, 2 out of 6) or an in-frame deletion (957-962del, 4 out of 6). The fruR product regulates expression of the fruBKA operon, and FPr (encoded by fruB) can substitute for HPr (encoded by ptsH) (Saier et al. 1996). When the fruB gene was removed from JK30, the resulting strain JK31 had very slow glucose uptake when grown in a M9 minimal medium containing 6 g/L of glucose and 4 g/L of xylose (FIG. **2**D), and did not grow in a M9 minimal medium containing 10 g/L of glucose. A culture of JK31 cells were plated on M9-glucose agar plates and placed in an incubator at 37° C., and colonies were found and isolated after two days. These cells were

able to grow in a M9 minimal medium containing 10 g/L of glucose, and one isolate was designated as JK32.

Adaptive Evolution of Co-Utilization Strains in Minimal Media

The strains JK20 and JK32 were adaptively evolved in M9 minimal media to improve the conversion of sugars to ethanol. Cells were first adaptively evolved in a medium containing 10 g/L of glucose, and subsequently in a medium containing 6 g/L of glucose and 4 g/L of xylose. Next, cells were adaptively evolved in a medium containing 10 g/L of glucose and 1%-5% (v/v) ethanol, where the ethanol concentration was increased by 1% at each transfer. At 5% ethanol, the growth inhibition was significant and cells were again adaptively evolved in a medium containing 10 g/L of glucose and 5% ethanol. The resulting strains were designated as JK20E and JK32E, respectively.

We grew the initial and evolved strains in shake flasks containing a synthetic hydrolysate medium without known inhibitors from pretreatment (SynH) to evaluate their ability to convert high concentration of glucose and xylose to ethanol. An additional copy of PET cassette on a plasmid (pPET) was inserted into the initial strains and single isolates from the evolved strains to further increase the conversion of pyruvate to ethanol. Both the initial and evolved strains simultaneously consumed glucose and xylose and produced ethanol. While JK20E pPET did not show significant improvements over JK20 pPET (FIGS. **3**A and **3**B), we found that JK32E pPET had 2-fold increase in glucose uptake and improved ethanol production when compared to JK32 pPET (FIGS. **3**C and **3**D). The control strain JK10 pPET rapidly consumed glucose first and did not utilize much xylose after the glucose was depleted (FIG. **3**E). We found that JK32E pPET also produced significant amount of succinate in addition to ethanol (FIG. **3**D), and constructed a new strain JK33 pPET by deleting frdA gene from JK32E to remove succinate production. A summary of glucose and xylose uptake rates and succinate and ethanol production rates for each of JK10 pPET, JK20 pPET, JK20E pPET, JK32 pPET, and JK32E pPET is shown in FIG. **3**F.

Fermentation in Synthetic and AFEX-Treated Hydrolysate Media

The control strain JK10 pPET and the evolved strains JK32E pPET and JK33 pPET were grown in bioreactors with 100 ml working volumes to demonstrate the evolved strain's ability to co-utilize glucose and xylose in industrially relevant conditions. When grown in SynH using bioreactors, JK10 pPET reached a higher cell density (OD600 ~7) in 24 hours (compared to shake flasks, FIG. **3**E), consumed all glucose within 36 hours, and slowly consumed xylose afterwards (FIG. **4**A). JK32E pPET also reached a high cell density (OD600 ~7) in 36 hours, consumed all glucose within 48 hours, and consumed 92.5% of xylose within 72 hours (FIG. **4**B). JK33 pPET reached slightly lower cell density (OD600 ~5) in 48 hours, consumed all glucose within 60 hours, and consumed 90.6% of xylose within 132 hours (FIG. **4**C). Since the evaporation of ethanol from bioreactors due to sparging was significant, we estimated the amount of ethanol lost via evaporation using a simple mass transfer model. The amount lost was added to the measured ethanol titers yielding an estimated ethanol concentration (represented by dotted lines in FIGS. **4**A-**4**I).

The growth rate and cell density were lower in AFEX-pretreated corn stover and switchgrass hydrolysates (ACSH and ASGH) than in SynH due to the presence of inhibitory compounds from the pretreatment. In ACSH, JK10 pPET was able to utilize glucose within 48 hours, but did not utilize xylose after glucose was depleted (FIG. **4**D). JK32E pPET and JK33 pPET were able to co-utilize glucose and xylose, but the conversion slowed down after cells entered stationary phase, likely due to the additional stress caused by lignotoxins and/or accumulated ethanol (FIGS. **4**E and **4**F). The apparent stress from the pretreatment seemed less significant in ASGH than ACSH. JK10 pPET consumed all glucose and slowly utilized xylose in this medium (FIG. **4**G). JK32E pPET and JK33 pPET were able to utilize all glucose and 93% and 71% of xylose, respectively (FIGS. **4**H and **4**I). The observed ethanol concentration was the highest for the JK33 pPET grown in ASGH among the all strains grown in ACSH or ASGH (Table 3). The estimated amount of ethanol produced by JK33 pPET in ASGH was 900.4 mM (41.5 g/L) which is 109.8% of the theoretical yield based on the consumed glucose and xylose, which could be explained by the other sugars consumed in ACSH and ASGH (e.g., arabinose, fructose, and galactose).

TABLE 3

Sugar consumption and ethanol production in hydrolysate media

				Produced (mM)		Yield (%)
Media	Consumed (mM)				Ethanol	Ethanol
Strains	Glucose	Xylose	Pyruvate	Succinate	(Estimated)	(Estimated)
SynH						
JK10 pPET	353.3	199.3	27.4	41.0	522.4 (758.0)	60.9 (72.9)
JK32E pPET	354.3	235.2	0.0	51.4	670.2 (850.3)	61.6 (77.3)
JK33 pPET	348.5	235.1	0.0	1.7	645.2 (1040.9)	65.8 (95.6)
ACSH						
JK10 pPET	375.9	21.5	0.0	60.2	494.1 (611.8)	64.4 (78.7)
JK32E pPET	311.1	126.3	23.3	74.3	330.6 (572.2)	51.5 (68.7)
JK33 pPET	225.0	88.0	0.0	0.5	390.0 (597.5)	75.2 (100.1)
ASGH						
JK10 pPET	344.8	116.5	31.0	76.0	415.9 (675.6)	55.8 (76.4)
JK32E pPET	347.7	205.7	11.5	100.7	443.7 (787.5)	45.7 (75.8)
JK33 pPET	292.3	141.0	0.0	0.1	526.8 (900.4)	69.2 (109.8)

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SEQUENCE LISTING

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<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

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gaacgttttc tgagcggtcg tgccaaggca tcagcccagc tggaaacgat caaaacgaaa 180

gctggtgaaa cgttcggtga agaaaaagaa gccatctttg aagggcatat tatgctgctc 240

gaagatgagg agctggagca ggaaatcata gccctgatta aagataagca catgacagct 300

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gctgacctga cgccgtccga aaccgcacag ctgaacctga agaaggtgct gggtttcatc 540

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gacgaattct ctatgagcgc catttctatc ccgcgcatta agaagattat ccgtaacacg 1620

aacttcgaag atgcgaaggt gttagcagag caggctcttg ctcaaccgac aacggacgag 1680

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<213> ORGANISM: Escherichia coli

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20 25 30

Ala Asp Gln Val Asp Gln Glu Val Glu Arg Phe Leu Ser Gly Arg Ala

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        35                  40                  45

Lys Ala Ser Ala Gln Leu Glu Thr Ile Lys Thr Lys Ala Gly Glu Thr
    50                  55                  60

Phe Gly Glu Glu Lys Glu Ala Ile Phe Glu Gly His Ile Met Leu Leu
65                  70                  75                  80

Glu Asp Glu Glu Leu Glu Gln Glu Ile Ile Ala Leu Ile Lys Asp Lys
                85                  90                  95

His Met Thr Ala Asp Ala Ala Ala His Glu Val Ile Glu Gly Gln Ala
            100                 105                 110

Ser Ala Leu Glu Glu Leu Asp Asp Glu Tyr Leu Lys Glu Arg Ala Ala
        115                 120                 125

Asp Val Arg Asp Ile Gly Lys Arg Leu Leu Arg Asn Ile Leu Gly Leu
    130                 135                 140

Lys Ile Ile Asp Leu Ser Ala Ile Gln Asp Glu Val Ile Leu Val Ala
145                 150                 155                 160

Ala Asp Leu Thr Pro Ser Glu Thr Ala Gln Leu Asn Leu Lys Lys Val
                165                 170                 175

Leu Gly Phe Ile Thr Asp Ala Gly Gly Arg Thr Ser His Thr Ser Ile
            180                 185                 190

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

Thr Ser Gln Val Lys Asn Asp Asp Tyr Leu Ile Leu Asp Ala Val Asn
    210                 215                 220

Asn Gln Val Tyr Val Asn Pro Thr Asn Glu Val Ile Asp Lys Met Arg
225                 230                 235                 240

Ala Val Gln Glu Gln Val Ala Ser Glu Lys Ala Glu Leu Ala Lys Leu
                245                 250                 255

Lys Asp Leu Pro Ala Ile Thr Leu Asp Gly His Gln Val Glu Val Cys
            260                 265                 270

Ala Asn Ile Gly Thr Val Arg Asp Val Glu Gly Ala Glu Arg Asn Gly
        275                 280                 285

Ala Glu Gly Val Gly Leu Tyr Arg Thr Glu Phe Leu Phe Met Asp Arg
    290                 295                 300

Asp Ala Leu Pro Thr Glu Glu Glu Gln Phe Ala Ala Tyr Lys Ala Val
305                 310                 315                 320

Ala Glu Ala Cys Gly Ser Gln Ala Val Ile Val Arg Thr Met Asp Ile
                325                 330                 335

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

Pro Phe Leu Gly Trp Arg Ala Ile Arg Ile Ala Met Asp Arg Arg Glu
        355                 360                 365

Ile Leu Arg Asp Gln Leu Arg Ala Ile Leu Arg Ala Ser Ala Phe Gly
    370                 375                 380

Lys Leu Arg Ile Met Phe Pro Met Ile Ile Ser Val Glu Glu Val Arg
385                 390                 395                 400

Ala Leu Arg Lys Glu Ile Glu Ile Tyr Lys Gln Glu Leu Arg Asp Glu
                405                 410                 415

Gly Lys Ala Phe Asp Glu Ser Ile Glu Ile Gly Val Met Val Glu Thr
            420                 425                 430

Pro Ala Ala Ala Thr Ile Ala Arg His Leu Ala Lys Glu Val Asp Phe
        435                 440                 445

Phe Ser Ile Gly Thr Asn Asp Leu Thr Gln Tyr Thr Leu Ala Val Asp
    450                 455                 460
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Arg Gly Asn Asp Met Ile Ser His Leu Tyr Gln Pro Met Ser Pro Ser
465                 470                 475                 480

Val Leu Asn Leu Ile Lys Gln Val Ile Asp Ala Ser His Ala Glu Gly
                485                 490                 495

Lys Trp Thr Gly Met Cys Gly Glu Leu Ala Gly Asp Glu Arg Ala Thr
            500                 505                 510

Leu Leu Leu Leu Gly Met Gly Leu Asp Glu Phe Ser Met Ser Ala Ile
        515                 520                 525

Ser Ile Pro Arg Ile Lys Lys Ile Ile Arg Asn Thr Asn Phe Glu Asp
    530                 535                 540

Ala Lys Val Leu Ala Glu Gln Ala Leu Ala Gln Pro Thr Thr Asp Glu
545                 550                 555                 560

Leu Met Thr Leu Val Asn Lys Phe Ile Glu Glu Lys Thr Ile Cys
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agcgccagcg cgaaaagcct gtttaaactg cagactctgg gcctgactca aggtaccgtt    180

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Arg Pro Ala Ala Gln Phe Val Lys Glu Ala Lys Gly Phe Thr Ser Glu
            20                  25                  30

Ile Thr Val Thr Ser Asn Gly Lys Ser Ala Ser Ala Lys Ser Leu Phe
        35                  40                  45

Lys Leu Gln Thr Leu Gly Leu Thr Gln Gly Thr Val Val Thr Ile Ser
    50                  55                  60

Ala Glu Gly Glu Asp Glu Gln Lys Ala Val Glu His Leu Val Lys Leu
65                  70                  75                  80

Met Ala Glu Leu Glu
                85


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<213> ORGANISM: Escherichia coli

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aatggcatgc tggcgcgcga acagcaaacc tcaacgttcc tcggcaatgg tattgctatt    180
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gcaaatgctt ttgatgtgga cggcgaaacg gcagccatgc tggtgagtgt ggcgatgaat    720

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aaccttgatg gtaccggcaa accggcaaac ggacgtagtc tgatgaaagt tgtggcactt   1020

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Asp Lys Glu Glu Ala Ile Arg Gln Val Ala Ala Ala Leu Val Gln Ala
            20                  25                  30

Gly Asn Val Ala Glu Gly Tyr Val Asn Gly Met Leu Ala Arg Glu Gln
        35                  40                  45

Gln Thr Ser Thr Phe Leu Gly Asn Gly Ile Ala Ile Pro His Gly Thr
    50                  55                  60

Thr Asp Thr Arg Asp Gln Val Leu Lys Thr Gly Val Gln Val Phe Gln
65                  70                  75                  80

Phe Pro Glu Gly Val Thr Trp Gly Asp Gly Gln Val Ala Tyr Val Ala
                85                  90                  95

Ile Gly Ile Ala Ala Ser Ser Asp Glu His Leu Gly Leu Leu Arg Gln
            100                 105                 110

Leu Thr His Val Leu Ser Asp Asp Ser Val Ala Glu Gln Leu Lys Ser
        115                 120                 125

Ala Thr Thr Ala Glu Glu Leu Arg Ala Leu Leu Met Gly Glu Lys Gln
    130                 135                 140

Ser Glu Gln Leu Lys Leu Asp Asn Glu Met Leu Thr Leu Asp Ile Val
145                 150                 155                 160

Ala Ser Asp Leu Leu Thr Leu Gln Ala Leu Asn Ala Ala Arg Leu Lys
                165                 170                 175

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

Gln Pro Leu Asn Leu Gly Gln Gly Ile Trp Leu Ser Asp Ser Ala Glu
        195                 200                 205
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Gly Asn Leu Arg Ser Ala Ile Ala Val Ser Arg Ala Ala Asn Ala Phe
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Asp Val Asp Gly Glu Thr Ala Ala Met Leu Val Ser Val Ala Met Asn
225                 230                 235                 240

Asp Asp Gln Pro Ile Ala Val Leu Lys Arg Leu Ala Asp Leu Leu Leu
                245                 250                 255

Asp Asn Lys Ala Asp Arg Leu Leu Lys Ala Asp Ala Ala Thr Leu Leu
            260                 265                 270

Ala Leu Leu Thr Ser Asp Asp Ala Pro Thr Asp Asp Val Leu Ser Ala
        275                 280                 285

Glu Phe Val Val Arg Asn Glu His Gly Leu His Ala Arg Pro Gly Thr
    290                 295                 300

Met Leu Val Asn Thr Ile Lys Gln Phe Asn Ser Asp Ile Thr Val Thr
305                 310                 315                 320

Asn Leu Asp Gly Thr Gly Lys Pro Ala Asn Gly Arg Ser Leu Met Lys
                325                 330                 335

Val Val Ala Leu Gly Val Lys Lys Gly His Arg Leu Arg Phe Thr Ala
            340                 345                 350

Gln Gly Ala Asp Ala Glu Gln Ala Leu Lys Ala Ile Gly Asp Ala Ile
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Ala Ala Gly Leu Gly Glu Gly Ala
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<213> ORGANISM: Escherichia coli

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gtcgcgccag tagacggcac cattggtaaa atctttgaaa ccaaccacgc attctctatc    240

gaatctgata gcggcgttga actgttcgtc cacttcggta tcgacaccgt tgaactgaaa    300

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            20                  25                  30

Asn Ile Glu Asp Val Pro Asp Val Val Phe Ala Glu Lys Ile Val Gly
        35                  40                  45

Asp Gly Ile Ala Ile Lys Pro Thr Gly Asn Lys Met Val Ala Pro Val
    50                  55                  60
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Asp Gly Thr Ile Gly Lys Ile Phe Glu Thr Asn His Ala Phe Ser Ile
65                  70                  75                  80

Glu Ser Asp Ser Gly Val Glu Leu Phe Val His Phe Gly Ile Asp Thr
                85                  90                  95

Val Glu Leu Lys Gly Glu Gly Phe Lys Arg Ile Ala Glu Glu Gly Gln
            100                 105                 110

Arg Val Lys Val Gly Asp Thr Val Ile Glu Phe Asp Leu Pro Leu Leu
        115                 120                 125

Glu Glu Lys Ala Lys Ser Thr Leu Thr Pro Val Val Ile Ser Asn Met
    130                 135                 140

Asp Glu Ile Lys Glu Leu Ile Lys Leu Ser Gly Ser Val Thr Val Gly
145                 150                 155                 160

Glu Thr Pro Val Ile Arg Ile Lys Lys
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<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

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cccgccgttg tatcgcatgt tatggcagaa gcaggcggtt ccgtctttgc aaacatgcca    180

ctgatttttg cgatcggtgt cgccctcggc tttaccaata acgatggcgt atccgcgctg    240

gctgcagttg ttgcctatgg catcatggtt aaaaccatgg ccgtggttgc gccactggta    300

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cctgagtatc ttggcttctt tgccggtaaa cgctttgtgc cgatcatttc tggcctggct    480

gccatcttta ctggcgttgt gctgtccttc atttggccgc cgattggttc tgcaatccag    540

accttctctc agtgggctgc ttaccagaac ccggtagttg cgtttggcat ttacggtttc    600

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cagattggtg aatacaccaa cgcagcaggt caggttttcc acggcgacat tccgcgttat    720

atggcgggtg acccgactgc gggtaaactg tctggtggct tcctgttcaa aatgtacggt    780

ctgccagctg ccgcaattgc tatctggcac tctgctaaac cagaaaaccg cgcgaaagtg    840

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tgtattaccc gtctgcgcgt cagcgttgct gatgtgtcta aagtggatca ggctggcctg   1320

aagaaactgg gcgcagcggg cgtagtggtt gctggttctg gtgttcaggc gattttcggt   1380

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20 25 30

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

Ala Glu Ala Gly Gly Ser Val Phe Ala Asn Met Pro Leu Ile Phe Ala
50 55 60

Ile Gly Val Ala Leu Gly Phe Thr Asn Asn Asp Gly Val Ser Ala Leu
65 70 75 80

Ala Ala Val Val Ala Tyr Gly Ile Met Val Lys Thr Met Ala Val Val
85 90 95

Ala Pro Leu Val Leu His Leu Pro Ala Glu Glu Ile Ala Ser Lys His
100 105 110

Leu Ala Asp Thr Gly Val Leu Gly Gly Ile Ile Ser Gly Ala Ile Ala
115 120 125

Ala Tyr Met Phe Asn Arg Phe Tyr Arg Ile Lys Leu Pro Glu Tyr Leu
130 135 140

Gly Phe Phe Ala Gly Lys Arg Phe Val Pro Ile Ile Ser Gly Leu Ala
145 150 155 160

Ala Ile Phe Thr Gly Val Val Leu Ser Phe Ile Trp Pro Pro Ile Gly
165 170 175

Ser Ala Ile Gln Thr Phe Ser Gln Trp Ala Ala Tyr Gln Asn Pro Val
180 185 190

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

Gly Leu His His Ile Trp Asn Val Pro Phe Gln Met Gln Ile Gly Glu
210 215 220

Tyr Thr Asn Ala Ala Gly Gln Val Phe His Gly Asp Ile Pro Arg Tyr
225 230 235 240

Met Ala Gly Asp Pro Thr Ala Gly Lys Leu Ser Gly Gly Phe Leu Phe
245 250 255

Lys Met Tyr Gly Leu Pro Ala Ala Ala Ile Ala Ile Trp His Ser Ala
260 265 270

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

Leu Thr Ser Phe Leu Thr Gly Ile Thr Glu Pro Ile Glu Phe Ser Phe
290 295 300

Met Phe Val Ala Pro Ile Leu Tyr Ile Ile His Ala Ile Leu Ala Gly
305 310 315 320

Leu Ala Phe Pro Ile Cys Ile Leu Leu Gly Met Arg Asp Gly Thr Ser
325 330 335

Phe Ser His Gly Leu Ile Asp Phe Ile Val Leu Ser Gly Asn Ser Ser
340 345 350

Lys Leu Trp Leu Phe Pro Ile Val Gly Ile Gly Tyr Ala Ile Val Tyr
355 360 365

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

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Gly Arg Glu Asp Ala Thr Glu Asp Ala Lys Ala Thr Gly Thr Ser Glu
385                 390                 395                 400

Met Ala Pro Ala Leu Val Ala Ala Phe Gly Gly Lys Glu Asn Ile Thr
                405                 410                 415

Asn Leu Asp Ala Cys Ile Thr Arg Leu Arg Val Ser Val Ala Asp Val
            420                 425                 430

Ser Lys Val Asp Gln Ala Gly Leu Lys Lys Leu Gly Ala Ala Gly Val
        435                 440                 445

Val Val Ala Gly Ser Gly Val Gln Ala Ile Phe Gly Thr Lys Ser Asp
    450                 455                 460

Asn Leu Lys Thr Glu Met Asp Glu Tyr Ile Arg Asn His
465                 470                 475


<210> SEQ ID NO 11
<211> LENGTH: 1650
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 11

atgaaaaaca tcaatccaac gcagaccgct gcctggcagg cactacagaa acacttcgat     60

gaaatgaaag acgttacgat cgccgatctt tttgctaaag acggcgatcg tttttctaag    120

ttctccgcaa ccttcgacga tcagatgctg gtggattact ccaaaaaccg catcactgaa    180

gagacgctgg cgaaattaca ggatctggcg aaagagtgcg atctggcggg cgcgattaag    240

tcgatgttct ctggcgagaa gatcaaccgc actgaaaacc gcgccgtgct gcacgtagcg    300

ctgcgtaacc gtagcaatac cccgattttg gttgatggca aagacgtaat gccggaagtc    360

aacgcggtgc tggagaagat gaaaaccttc tcagaagcga ttatttccgg tgagtggaaa    420

ggttataccg gcaaagcaat cactgacgta gtgaacatcg ggatcggcgg ttctgacctc    480

ggcccataca tggtgaccga agctctgcgt ccgtacaaaa accacctgaa catgcacttt    540

gtttctaacg tcgatgggac tcacatcgcg gaagtgctga aaaaagtaaa cccggaaacc    600

acgctgttct tggtagcatc taaaaccttc accactcagg aaactatgac caacgcccat    660

agcgcgcgtg actggttcct gaaagcggca ggtgatgaaa aacacgttgc aaaacacttt    720

gcggcgcttt ccaccaatgc caaagccgtt ggcgagtttg gtattgatac tgccaacatg    780

ttcgagttct gggactgggt tggcggccgt tactctttgt ggtcagcgat tggcctgtcg    840

attgttctct ccatcggctt tgataacttc gttgaactgc tttccggcgc acacgcgatg    900

gacaagcatt tctccaccac gcctgccgag aaaaacctgc ctgtactgct ggcgctgatt    960

ggcatctggt acaacaattt ctttggtgcg gaaactgaag cgattctgcc gtatgaccag   1020

tatatgcacc gtttcgcggc gtacttccag cagggcaata tggagtccaa cggtaagtat   1080

gttgaccgta acggtaacgt tgtggattac cagactggcc cgattatctg gggtgaacca   1140

ggcactaacg gtcagcacgc gttctaccag ctgatccacc agggaaccaa aatggtaccg   1200

tgcgatttca tcgctccggc tatcacccat aacccgctct ctgatcatca ccagaaactg   1260

ctgtctaact tcttcgccca gaccgaagcg ctggcgtttg gtaaatcccg cgaagtggtt   1320

gagcaggaat atcgtgatca gggtaaagat ccggcaacgc ttgactacgt ggtgccgttc   1380

aaagtattcg aaggtaaccg cccgaccaac tccatcctgc tgcgtgaaat cactccgttc   1440

agcctgggtg cgttgattgc gctgtatgag cacaaaatct ttactcaggg cgtgatcctg   1500

aacatcttca ccttcgacca gtggggcgtg gaactgggta aacagctggc gaaccgtatt   1560

ctgccagagc tgaaagatga taaagaaatc agcagccacg atagctcgac caatggtctg   1620
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attaaccgct ataaagcgtg gcgcggttaa 1650

<210> SEQ ID NO 12
<211> LENGTH: 549
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 12

Met Lys Asn Ile Asn Pro Thr Gln Thr Ala Ala Trp Gln Ala Leu Gln
1 5 10 15

Lys His Phe Asp Glu Met Lys Asp Val Thr Ile Ala Asp Leu Phe Ala
20 25 30

Lys Asp Gly Asp Arg Phe Ser Lys Phe Ser Ala Thr Phe Asp Asp Gln
35 40 45

Met Leu Val Asp Tyr Ser Lys Asn Arg Ile Thr Glu Glu Thr Leu Ala
50 55 60

Lys Leu Gln Asp Leu Ala Lys Glu Cys Asp Leu Ala Gly Ala Ile Lys
65 70 75 80

Ser Met Phe Ser Gly Glu Lys Ile Asn Arg Thr Glu Asn Arg Ala Val
85 90 95

Leu His Val Ala Leu Arg Asn Arg Ser Asn Thr Pro Ile Leu Val Asp
100 105 110

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

Thr Phe Ser Glu Ala Ile Ile Ser Gly Glu Trp Lys Gly Tyr Thr Gly
130 135 140

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

Gly Pro Tyr Met Val Thr Glu Ala Leu Arg Pro Tyr Lys Asn His Leu
165 170 175

Asn Met His Phe Val Ser Asn Val Asp Gly Thr His Ile Ala Glu Val
180 185 190

Leu Lys Lys Val Asn Pro Glu Thr Thr Leu Phe Leu Val Ala Ser Lys
195 200 205

Thr Phe Thr Thr Gln Glu Thr Met Thr Asn Ala His Ser Ala Arg Asp
210 215 220

Trp Phe Leu Lys Ala Ala Gly Asp Glu Lys His Val Ala Lys His Phe
225 230 235 240

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

Thr Ala Asn Met Phe Glu Phe Trp Asp Trp Val Gly Gly Arg Tyr Ser
260 265 270

Leu Trp Ser Ala Ile Gly Leu Ser Ile Val Leu Ser Ile Gly Phe Asp
275 280 285

Asn Phe Val Glu Leu Leu Ser Gly Ala His Ala Met Asp Lys His Phe
290 295 300

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

Gly Ile Trp Tyr Asn Asn Phe Phe Gly Ala Glu Thr Glu Ala Ile Leu
325 330 335

Pro Tyr Asp Gln Tyr Met His Arg Phe Ala Ala Tyr Phe Gln Gln Gly
340 345 350

Asn Met Glu Ser Asn Gly Lys Tyr Val Asp Arg Asn Gly Asn Val Val
355 360 365

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Asp Tyr Gln Thr Gly Pro Ile Ile Trp Gly Glu Pro Gly Thr Asn Gly
    370                 375                 380

Gln His Ala Phe Tyr Gln Leu Ile His Gln Gly Thr Lys Met Val Pro
385                 390                 395                 400

Cys Asp Phe Ile Ala Pro Ala Ile Thr His Asn Pro Leu Ser Asp His
                405                 410                 415

His Gln Lys Leu Leu Ser Asn Phe Phe Ala Gln Thr Glu Ala Leu Ala
            420                 425                 430

Phe Gly Lys Ser Arg Glu Val Val Glu Gln Glu Tyr Arg Asp Gln Gly
        435                 440                 445

Lys Asp Pro Ala Thr Leu Asp Tyr Val Val Pro Phe Lys Val Phe Glu
    450                 455                 460

Gly Asn Arg Pro Thr Asn Ser Ile Leu Leu Arg Glu Ile Thr Pro Phe
465                 470                 475                 480

Ser Leu Gly Ala Leu Ile Ala Leu Tyr Glu His Lys Ile Phe Thr Gln
                485                 490                 495

Gly Val Ile Leu Asn Ile Phe Thr Phe Asp Gln Trp Gly Val Glu Leu
            500                 505                 510

Gly Lys Gln Leu Ala Asn Arg Ile Leu Pro Glu Leu Lys Asp Asp Lys
        515                 520                 525

Glu Ile Ser Ser His Asp Ser Ser Thr Asn Gly Leu Ile Asn Arg Tyr
    530                 535                 540

Lys Ala Trp Arg Gly
545
```

<210> SEQ ID NO 13
<211> LENGTH: 996
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 13

```
atgaaaaaga aaagacccgt acttcaggat gtggctgacc gtgtaggcgt gaccaaaatg     60

acggtcagcc gttttttacg caacccggag caggtttccg tcgctctacg cggcaagatt    120

gccgcggctc ttgatgaact gggctatatt cccaatcgtg cgcccgatat cctctctaac    180

gccaccagcc gggcgattgg cgtcctgtta ccttctctca ccaaccaggt tttcgcggaa    240

gtattacgcg gaatcgaaag cgtcaccgac gcgcacggtt atcagaccat gctggcgcac    300

tacggttata aaccggaaat ggagcaagaa cgcctcgaat ccatgctctc ctggaatatc    360

gacggcctga tcctcaccga acgtacccac acgccgcgca ccttaaagat gattgaagtg    420

gcgggtattc ccgtggtgga actgatggac agcaagtcgc catgccttga tatcgccgtc    480

ggttttgata actttgaagc agcacgccag atgaccactg ccattattgc tcgcgggcat    540

cgccacattg cctatctcgg cgcacgtctc gacgaacgta ctatcatcaa acagaaggga    600

tacgaacagg cgatgctgga tgcaggcctg gtgccatata gcgtgatggt tgagcaatct    660

tcttcttact cttccggtat tgaactgatt cgccaggcgc ggcgggaata tccgcagctg    720

gatggcgtgt tctgtacgaa tgatgacctg gcggtcggcg cggcgtttga atgtcagcgt    780

ctggggttaa aagttcctga cgatatggcg attgccggtt tccacggtca tgacattggt    840

caggtgatgg agccacgact tgcgagcgtg ctgacgccgc gtgagcggat gggcagtatt    900

ggcgctgaac gcctgctggc gcgtattcgt ggcgaatctg tgacaccgaa aatgttagat    960

ttaggtttca ccttgtcacc gggcggatct atttaa                               996
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<210> SEQ ID NO 14
<211> LENGTH: 331
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 14

Met Lys Lys Lys Arg Pro Val Leu Gln Asp Val Ala Asp Arg Val Gly
1 5 10 15

Val Thr Lys Met Thr Val Ser Arg Phe Leu Arg Asn Pro Glu Gln Val
20 25 30

Ser Val Ala Leu Arg Gly Lys Ile Ala Ala Ala Leu Asp Glu Leu Gly
35 40 45

Tyr Ile Pro Asn Arg Ala Pro Asp Ile Leu Ser Asn Ala Thr Ser Arg
50 55 60

Ala Ile Gly Val Leu Leu Pro Ser Leu Thr Asn Gln Val Phe Ala Glu
65 70 75 80

Val Leu Arg Gly Ile Glu Ser Val Thr Asp Ala His Gly Tyr Gln Thr
85 90 95

Met Leu Ala His Tyr Gly Tyr Lys Pro Glu Met Glu Gln Glu Arg Leu
100 105 110

Glu Ser Met Leu Ser Trp Asn Ile Asp Gly Leu Ile Leu Thr Glu Arg
115 120 125

Thr His Thr Pro Arg Thr Leu Lys Met Ile Glu Val Ala Gly Ile Pro
130 135 140

Val Val Glu Leu Met Asp Ser Lys Ser Pro Cys Leu Asp Ile Ala Val
145 150 155 160

Gly Phe Asp Asn Phe Glu Ala Ala Arg Gln Met Thr Thr Ala Ile Ile
165 170 175

Ala Arg Gly His Arg His Ile Ala Tyr Leu Gly Ala Arg Leu Asp Glu
180 185 190

Arg Thr Ile Ile Lys Gln Lys Gly Tyr Glu Gln Ala Met Leu Asp Ala
195 200 205

Gly Leu Val Pro Tyr Ser Val Met Val Glu Gln Ser Ser Ser Tyr Ser
210 215 220

Ser Gly Ile Glu Leu Ile Arg Gln Ala Arg Arg Glu Tyr Pro Gln Leu
225 230 235 240

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

Glu Cys Gln Arg Leu Gly Leu Lys Val Pro Asp Asp Met Ala Ile Ala
260 265 270

Gly Phe His Gly His Asp Ile Gly Gln Val Met Glu Pro Arg Leu Ala
275 280 285

Ser Val Leu Thr Pro Arg Glu Arg Met Gly Ser Ile Gly Ala Glu Arg
290 295 300

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

Leu Gly Phe Thr Leu Ser Pro Gly Gly Ser Ile
325 330

<210> SEQ ID NO 15
<211> LENGTH: 990
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 15

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atgaaactcg ccgtttatag cacaaaacag tacgacaaga agtacctgca acaggtgaac     60

gagtcctttg gctttgagct ggaatttttt gactttctgc tgacggaaaa aaccgctaaa    120

actgccaatg gctgcgaagc ggtatgtatt ttcgtaaacg atgacggcag ccgcccggtg    180

ctggaagagc tgaaaaagca cggcgttaaa tatatcgccc tgcgctgtgc cggtttcaat    240

aacgtcgacc ttgacgcggc aaaagaactg gggctgaaag tagtccgtgt tccagcctat    300

gatccagagg ccgttgctga acacgccatc ggtatgatga tgacgctgaa ccgccgtatt    360

caccgcgcgt atcagcgtac ccgtgatgct aacttctctc tggaaggtct gaccggcttt    420

actatgtatg gcaaaacggc aggcgttatc ggtaccggta aaatcggtgt ggcgatgctg    480

cgcattctga aaggttttgg tatgcgtctg ctggcgttcg atccgtatcc aagtgcagcg    540

gcgctggaac tcggtgtgga gtatgtcgat ctgccaaccc tgttctctga atcagacgtt    600

atctctctgc actgcccgct gacaccggaa aactatcatc tgttgaacga agccgccttc    660

gaacagatga aaaatggcgt gatgatcgtc aataccagtc gcggtgcatt gattgattct    720

caggcagcaa ttgaagcgct gaaaaatcag aaaattggtt cgttgggtat ggacgtgtat    780

gagaacgaac gcgatctatt ctttgaagat aaatccaacg acgtgatcca ggatgacgta    840

ttccgtcgcc tgtctgcctg ccacaacgtg ctgtttaccg ggcaccaggc attcctgaca    900

gcagaagctc tgaccagtat ttctcagact acgctgcaaa acttaagcaa tctggaaaaa    960

ggcgaaacct gcccgaacga actggtttaa                                     990


<210> SEQ ID NO 16
<211> LENGTH: 329
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 16

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

Gln Gln Val Asn Glu Ser Phe Gly Phe Glu Leu Glu Phe Phe Asp Phe
            20                  25                  30

Leu Leu Thr Glu Lys Thr Ala Lys Thr Ala Asn Gly Cys Glu Ala Val
        35                  40                  45

Cys Ile Phe Val Asn Asp Asp Gly Ser Arg Pro Val Leu Glu Glu Leu
    50                  55                  60

Lys Lys His Gly Val Lys Tyr Ile Ala Leu Arg Cys Ala Gly Phe Asn
65                  70                  75                  80

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

Val Pro Ala Tyr Asp Pro Glu Ala Val Ala Glu His Ala Ile Gly Met
            100                 105                 110

Met Met Thr Leu Asn Arg Arg Ile His Arg Ala Tyr Gln Arg Thr Arg
        115                 120                 125

Asp Ala Asn Phe Ser Leu Glu Gly Leu Thr Gly Phe Thr Met Tyr Gly
    130                 135                 140

Lys Thr Ala Gly Val Ile Gly Thr Gly Lys Ile Gly Val Ala Met Leu
145                 150                 155                 160

Arg Ile Leu Lys Gly Phe Gly Met Arg Leu Leu Ala Phe Asp Pro Tyr
                165                 170                 175

Pro Ser Ala Ala Ala Leu Glu Leu Gly Val Glu Tyr Val Asp Leu Pro
            180                 185                 190

Thr Leu Phe Ser Glu Ser Asp Val Ile Ser Leu His Cys Pro Leu Thr
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        195                 200                 205

Pro Glu Asn Tyr His Leu Leu Asn Glu Ala Ala Phe Glu Gln Met Lys
    210                 215                 220

Asn Gly Val Met Ile Val Asn Thr Ser Arg Gly Ala Leu Ile Asp Ser
225                 230                 235                 240

Gln Ala Ala Ile Glu Ala Leu Lys Asn Gln Lys Ile Gly Ser Leu Gly
                245                 250                 255

Met Asp Val Tyr Glu Asn Glu Arg Asp Leu Phe Phe Glu Asp Lys Ser
            260                 265                 270

Asn Asp Val Ile Gln Asp Asp Val Phe Arg Arg Leu Ser Ala Cys His
        275                 280                 285

Asn Val Leu Phe Thr Gly His Gln Ala Phe Leu Thr Ala Glu Ala Leu
    290                 295                 300

Thr Ser Ile Ser Gln Thr Thr Leu Gln Asn Leu Ser Asn Leu Glu Lys
305                 310                 315                 320

Gly Glu Thr Cys Pro Asn Glu Leu Val
                325


<210> SEQ ID NO 17
<211> LENGTH: 2283
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 17

atgtccgagc ttaatgaaaa gttagccaca gcctgggaag gttttaccaa aggtgactgg     60

cagaatgaag taaacgtccg tgacttcatt cagaaaaact acactccgta cgagggtgac    120

gagtccttcc tggctggcgc tactgaagcg accaccaccc tgtgggacaa agtaatggaa    180

ggcgttaaac tggaaaaccg cactcacgcg ccagttgact ttgacaccgc tgttgcttcc    240

accatcacct ctcacgacgc tggctacatc aacaagcagc ttgagaaaat cgttggtctg    300

cagactgaag ctccgctgaa acgtgctctt atcccgttcg gtggtatcaa aatgatcgaa    360

ggttcctgca aagcgtacaa ccgcgaactg gatccgatga tcaaaaaaat cttcactgaa    420

taccgtaaaa ctcacaacca gggcgtgttc gacgtttaca ctccggacat cctgcgttgc    480

cgtaaatctg gtgttctgac cggtctgcca gatgcatatg gccgtggccg tatcatcggt    540

gactaccgtc gcgttgcgct gtacggtatc gactacctga tgaaagacaa actggcacag    600

ttcacttctc tgcaggctga tctggaaaac ggcgtaaacc tggaacagac tatccgtctg    660

cgcgaagaaa tcgctgaaca gcaccgcgct ctgggtcaga tgaaagaaat ggctgcgaaa    720

tacggctacg acatctctgg tccggctacc aacgctcagg aagctatcca gtggacttac    780

ttcggctacc tggctgctgt taagtctcag aacggtgctg caatgtcctt cggtcgtacc    840

tccaccttcc tggatgtgta catcgaacgt gacctgaaag ctggcaagat caccgaacaa    900

gaagcgcagg aaatggttga ccacctggtc atgaaactgc gtatggttcg cttcctgcgt    960

actccggaat acgatgaact gttctctggc gacccgatct gggcaaccga atctatcggt   1020

ggtatgggcc tcgacggtcg taccctggtt accaaaaaca gcttccgttt cctgaacacc   1080

ctgtacacca tgggtccgtc tccggaaccg aacatgacca ttctgtggtc tgaaaaactg   1140

ccgctgaact tcaagaaatt cgccgctaaa gtgtccatcg acacctcttc tctgcagtat   1200

gagaacgatg acctgatgcg tccggacttc aacaacgatg actacgctat tgcttgctgc   1260

gtaagcccga tgatcgttgg taaacaaatg cagttcttcg gtgcgcgtgc aaacctggcg   1320

aaaaccatgc tgtacgcaat caacggcggc gttgacgaaa aactgaaaat gcaggttggt   1380
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ccgaagtctg aaccgatcaa aggcgatgtc ctgaactatg atgaagtgat ggagcgcatg   1440

gatcacttca tggactggct ggctaaacag tacatcactg cactgaacat catccactac   1500

atgcacgaca agtacagcta cgaagcctct ctgatggcgc tgcacgaccg tgacgttatc   1560

cgcaccatgg cgtgtggtat cgctggtctg tccgttgctg ctgactccct gtctgcaatc   1620

aaatatgcga aagttaaacc gattcgtgac gaagacggtc tggctatcga cttcgaaatc   1680

gaaggcgaat acccgcagtt tggtaacaat gatccgcgtg tagatgacct ggctgttgac   1740

ctggtagaac gtttcatgaa gaaaattcag aaactgcaca cctaccgtga cgctatcccg   1800

actcagtctg ttctgaccat cacttctaac gttgtgtatg gtaagaaaac gggtaacacc   1860

ccagacggtc gtcgtgctgg cgcgccgttc ggaccgggtg ctaacccgat gcacggtcgt   1920

gaccagaaag gtgcagtagc ctctctgact tccgttgcta aactgccgtt tgcttacgct   1980

aaagatggta tctcctacac cttctctatc gttccgaacg cactgggtaa agacgacgaa   2040

gttcgtaaga ccaacctggc tggtctgatg gatggttact tccaccacga agcatccatc   2100

gaaggtggtc agcacctgaa cgttaacgtg atgaaccgtg aaatgctgct cgacgcgatg   2160

gaaaacccgg aaaaatatcc gcagctgacc atccgtgtat ctggctacgc agtacgtttc   2220

aactcgctga ctaaagaaca gcagcaggac gttattactc gtaccttcac tcaatctatg   2280

taa                                                                2283
```

<210> SEQ ID NO 18
<211> LENGTH: 760
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 18

```
Met Ser Glu Leu Asn Glu Lys Leu Ala Thr Ala Trp Glu Gly Phe Thr
1               5                   10                  15

Lys Gly Asp Trp Gln Asn Glu Val Asn Val Arg Asp Phe Ile Gln Lys
            20                  25                  30

Asn Tyr Thr Pro Tyr Glu Gly Asp Glu Ser Phe Leu Ala Gly Ala Thr
        35                  40                  45

Glu Ala Thr Thr Thr Leu Trp Asp Lys Val Met Glu Gly Val Lys Leu
    50                  55                  60

Glu Asn Arg Thr His Ala Pro Val Asp Phe Asp Thr Ala Val Ala Ser
65                  70                  75                  80

Thr Ile Thr Ser His Asp Ala Gly Tyr Ile Asn Lys Gln Leu Glu Lys
                85                  90                  95

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

Phe Gly Gly Ile Lys Met Ile Glu Gly Ser Cys Lys Ala Tyr Asn Arg
        115                 120                 125

Glu Leu Asp Pro Met Ile Lys Lys Ile Phe Thr Glu Tyr Arg Lys Thr
    130                 135                 140

His Asn Gln Gly Val Phe Asp Val Tyr Thr Pro Asp Ile Leu Arg Cys
145                 150                 155                 160

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

Arg Ile Ile Gly Asp Tyr Arg Arg Val Ala Leu Tyr Gly Ile Asp Tyr
            180                 185                 190

Leu Met Lys Asp Lys Leu Ala Gln Phe Thr Ser Leu Gln Ala Asp Leu
        195                 200                 205
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Glu Asn Gly Val Asn Leu Glu Gln Thr Ile Arg Leu Arg Glu Glu Ile
    210                 215                 220

Ala Glu Gln His Arg Ala Leu Gly Gln Met Lys Glu Met Ala Ala Lys
225                 230                 235                 240

Tyr Gly Tyr Asp Ile Ser Gly Pro Ala Thr Asn Ala Gln Glu Ala Ile
                245                 250                 255

Gln Trp Thr Tyr Phe Gly Tyr Leu Ala Ala Val Lys Ser Gln Asn Gly
            260                 265                 270

Ala Ala Met Ser Phe Gly Arg Thr Ser Thr Phe Leu Asp Val Tyr Ile
        275                 280                 285

Glu Arg Asp Leu Lys Ala Gly Lys Ile Thr Glu Gln Glu Ala Gln Glu
    290                 295                 300

Met Val Asp His Leu Val Met Lys Leu Arg Met Val Arg Phe Leu Arg
305                 310                 315                 320

Thr Pro Glu Tyr Asp Glu Leu Phe Ser Gly Asp Pro Ile Trp Ala Thr
                325                 330                 335

Glu Ser Ile Gly Gly Met Gly Leu Asp Gly Arg Thr Leu Val Thr Lys
            340                 345                 350

Asn Ser Phe Arg Phe Leu Asn Thr Leu Tyr Thr Met Gly Pro Ser Pro
        355                 360                 365

Glu Pro Asn Met Thr Ile Leu Trp Ser Glu Lys Leu Pro Leu Asn Phe
    370                 375                 380

Lys Lys Phe Ala Ala Lys Val Ser Ile Asp Thr Ser Ser Leu Gln Tyr
385                 390                 395                 400

Glu Asn Asp Asp Leu Met Arg Pro Asp Phe Asn Asn Asp Asp Tyr Ala
                405                 410                 415

Ile Ala Cys Cys Val Ser Pro Met Ile Val Gly Lys Gln Met Gln Phe
            420                 425                 430

Phe Gly Ala Arg Ala Asn Leu Ala Lys Thr Met Leu Tyr Ala Ile Asn
        435                 440                 445

Gly Gly Val Asp Glu Lys Leu Lys Met Gln Val Gly Pro Lys Ser Glu
    450                 455                 460

Pro Ile Lys Gly Asp Val Leu Asn Tyr Asp Glu Val Met Glu Arg Met
465                 470                 475                 480

Asp His Phe Met Asp Trp Leu Ala Lys Gln Tyr Ile Thr Ala Leu Asn
                485                 490                 495

Ile Ile His Tyr Met His Asp Lys Tyr Ser Tyr Glu Ala Ser Leu Met
            500                 505                 510

Ala Leu His Asp Arg Asp Val Ile Arg Thr Met Ala Cys Gly Ile Ala
        515                 520                 525

Gly Leu Ser Val Ala Ala Asp Ser Leu Ser Ala Ile Lys Tyr Ala Lys
    530                 535                 540

Val Lys Pro Ile Arg Asp Glu Asp Gly Leu Ala Ile Asp Phe Glu Ile
545                 550                 555                 560

Glu Gly Glu Tyr Pro Gln Phe Gly Asn Asn Asp Pro Arg Val Asp Asp
                565                 570                 575

Leu Ala Val Asp Leu Val Glu Arg Phe Met Lys Lys Ile Gln Lys Leu
            580                 585                 590

His Thr Tyr Arg Asp Ala Ile Pro Thr Gln Ser Val Leu Thr Ile Thr
        595                 600                 605

Ser Asn Val Val Tyr Gly Lys Lys Thr Gly Asn Thr Pro Asp Gly Arg
    610                 615                 620
```

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Arg Ala Gly Ala Pro Phe Gly Pro Gly Ala Asn Pro Met His Gly Arg
625                 630                 635                 640

Asp Gln Lys Gly Ala Val Ala Ser Leu Thr Ser Val Ala Lys Leu Pro
                645                 650                 655

Phe Ala Tyr Ala Lys Asp Gly Ile Ser Tyr Thr Phe Ser Ile Val Pro
            660                 665                 670

Asn Ala Leu Gly Lys Asp Asp Glu Val Arg Lys Thr Asn Leu Ala Gly
        675                 680                 685

Leu Met Asp Gly Tyr Phe His His Glu Ala Ser Ile Glu Gly Gly Gln
    690                 695                 700

His Leu Asn Val Asn Val Met Asn Arg Glu Met Leu Leu Asp Ala Met
705                 710                 715                 720

Glu Asn Pro Glu Lys Tyr Pro Gln Leu Thr Ile Arg Val Ser Gly Tyr
                725                 730                 735

Ala Val Arg Phe Asn Ser Leu Thr Lys Glu Gln Gln Gln Asp Val Ile
            740                 745                 750

Thr Arg Thr Phe Thr Gln Ser Met
        755                 760


<210> SEQ ID NO 19
<211> LENGTH: 741
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 19

atgtcagtta ttggtcgcat tcactccttt gaatcctgtg gaaccgtaga cggcccaggt     60

attcgcttta tcacctttt ccagggctgc ctgatgcgct gcctgtattg tcataaccgc    120

gacacctggg acacgcatgg cggtaaagaa gttaccgttg aagatttgat gaaggaagtg    180

gtgacctatc gccactttat gaacgcttcc ggcggcggcg ttaccgcatc cggcggtgaa    240

gcaatcctgc aagctgagtt tgttcgtgac tggttccgcg cctgcaaaaa agaaggcatt    300

catacctgtc tggacaccaa cggttttgtt cgtcgttacg atccggtgat tgatgaactg    360

ctggaagtaa ccgacctggt aatgctcgat ctcaaacaga tgaacgacga gatccaccaa    420

aatctggttg gagtttccaa ccaccgcacg ctggagttcg ctaaatatct ggcgaacaaa    480

aatgtgaagg tgtggatccg ctacgttgtt gtcccaggct ggtctgacga tgacgattca    540

gcgcatcgcc tcggtgaatt tacccgtgat atgggcaacg ttgagaaaat cgagcttctc    600

ccctaccacg agctgggcaa acacaaatgg gtggcaatgg gtgaagagta caaactcgac    660

ggtgttaaac caccgaagaa agagaccatg gaacgcgtga aaggcattct tgagcagtac    720

ggtcataagg taatgttcta a                                              741


<210> SEQ ID NO 20
<211> LENGTH: 246
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 20

Met Ser Val Ile Gly Arg Ile His Ser Phe Glu Ser Cys Gly Thr Val
1               5                   10                  15

Asp Gly Pro Gly Ile Arg Phe Ile Thr Phe Phe Gln Gly Cys Leu Met
            20                  25                  30

Arg Cys Leu Tyr Cys His Asn Arg Asp Thr Trp Asp Thr His Gly Gly
        35                  40                  45

Lys Glu Val Thr Val Glu Asp Leu Met Lys Glu Val Val Thr Tyr Arg
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    50                  55                  60

His Phe Met Asn Ala Ser Gly Gly Gly Val Thr Ala Ser Gly Gly Glu
65                  70                  75                  80

Ala Ile Leu Gln Ala Glu Phe Val Arg Asp Trp Phe Arg Ala Cys Lys
                85                  90                  95

Lys Glu Gly Ile His Thr Cys Leu Asp Thr Asn Gly Phe Val Arg Arg
            100                 105                 110

Tyr Asp Pro Val Ile Asp Glu Leu Leu Glu Val Thr Asp Leu Val Met
        115                 120                 125

Leu Asp Leu Lys Gln Met Asn Asp Glu Ile His Gln Asn Leu Val Gly
    130                 135                 140

Val Ser Asn His Arg Thr Leu Glu Phe Ala Lys Tyr Leu Ala Asn Lys
145                 150                 155                 160

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

Asp Asp Asp Ser Ala His Arg Leu Gly Glu Phe Thr Arg Asp Met Gly
            180                 185                 190

Asn Val Glu Lys Ile Glu Leu Leu Pro Tyr His Glu Leu Gly Lys His
        195                 200                 205

Lys Trp Val Ala Met Gly Glu Glu Tyr Lys Leu Asp Gly Val Lys Pro
    210                 215                 220

Pro Lys Lys Glu Thr Met Glu Arg Val Lys Gly Ile Leu Glu Gln Tyr
225                 230                 235                 240

Gly His Lys Val Met Phe
                245
```

<210> SEQ ID NO 21
<211> LENGTH: 1809
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 21

```
gtgcaaacct ttcaagccga tcttgccatt gtaggcgccg gtggcgcggg attacgtgct     60

gcaattgctg ccgcgcaggc aaatccgaat gcaaaaatcg cactaatctc aaaagtatac    120

ccgatgcgta gccataccgt tgctgcagaa gggggctccg ccgctgtcgc gcaggatcat    180

gacagcttcg aatatcactt tcacgataca gtagcgggtg gcgactggtt gtgtgagcag    240

gatgtcgtgg attatttcgt ccaccactgc ccaaccgaaa tgacccaact ggaactgtgg    300

ggatgcccat ggagccgtcg cccggatggt agcgtcaacg tacgtcgctt cggcggcatg    360

aaaatcgagc gcacctggtt cgccgccgat aagaccggct tccatatgct gcacacgctg    420

ttccagacct ctctgcaatt cccgcagatc cagcgttttg acgaacattt cgtgctggat    480

attctggttg atgatggtca tgttcgcggc ctggtagcaa tgaacatgat ggaaggcacg    540

ctggtgcaga tccgtgctaa cgcggtcgtt atggctactg gcggtgcggg tcgcgtttat    600

cgttacaaca ccaacggcgg catcgttacc ggtgacggta tgggtatggc gctaagccac    660

ggcgttccgc tgcgtgacat ggaattcgtt cagtatcacc caaccggtct gccaggttcc    720

ggtatcctga tgaccgaagg ttgccgcggt gaaggcggta ttctggtcaa caaaaatggc    780

taccgttatc tgcaagatta cggcatgggc ccggaaactc cgctgggcga gccgaaaaac    840

aaatatatgg aactgggtcc acgcgacaaa gtctctcagg ccttctggca cgaatggcgt    900

aaaggcaaca ccatctccac gccgcgtggc gatgtggttt atctcgactt gcgtcacctc    960

ggcgagaaaa aactgcatga acgtctgccg ttcatctgcg aactggcgaa agcgtacgtt   1020
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ggcgtcgatc cggttaaaga accgattccg gtacgtccga ccgcacacta caccatgggc   1080

ggtatcgaaa ccgatcagaa ctgtgaaacc cgcattaaag gtctgttcgc cgtgggtgaa   1140

tgttcctctg ttggtctgca cggtgcaaac cgtctgggtt ctaactccct ggcggaactg   1200

gtggtcttcg gccgtctggc cggtgaacaa gcgacagagc gtgcagcaac tgccggtaat   1260

ggcaacgaag cggcaattga agcgcaggca gctggcgttg aacaacgtct gaaagatctg   1320

gttaaccagg atggcggcga aaactgggcg aagatccgcg acgaaatggg cctggctatg   1380

gaagaaggct gcggtatcta ccgtacgccg gaactgatgc agaaaaccat cgacaagctg   1440

gcagagctgc aggaacgctt caagcgcgtg cgcatcaccg acacttccag cgtgttcaac   1500

accgacctgc tctacaccat tgaactgggc cacggtctga acgttgctga atgtatggcg   1560

cactccgcaa tggcacgtaa agagtcccgc ggcgcgcacc agcgtctgga cgaaggttgc   1620

accgagcgtg acgacgtcaa cttcctcaaa cacaccctcg ccttccgcga tgctgatggc   1680

acgactcgcc tggagtacag cgacgtgaag attactacgc tgccgccagc taaacgcgtt   1740

tacggtggcg aagcggatgc agccgataag gcggaagcag ccaataagaa ggagaaggcg   1800

aatggctga                                                          1809
```

<210> SEQ ID NO 22
<211> LENGTH: 602
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 22

```
Met Gln Thr Phe Gln Ala Asp Leu Ala Ile Val Gly Ala Gly Gly Ala
1               5                   10                  15

Gly Leu Arg Ala Ala Ile Ala Ala Ala Gln Ala Asn Pro Asn Ala Lys
            20                  25                  30

Ile Ala Leu Ile Ser Lys Val Tyr Pro Met Arg Ser His Thr Val Ala
        35                  40                  45

Ala Glu Gly Gly Ser Ala Ala Val Ala Gln Asp His Asp Ser Phe Glu
    50                  55                  60

Tyr His Phe His Asp Thr Val Ala Gly Gly Asp Trp Leu Cys Glu Gln
65                  70                  75                  80

Asp Val Val Asp Tyr Phe Val His His Cys Pro Thr Glu Met Thr Gln
                85                  90                  95

Leu Glu Leu Trp Gly Cys Pro Trp Ser Arg Arg Pro Asp Gly Ser Val
            100                 105                 110

Asn Val Arg Arg Phe Gly Gly Met Lys Ile Glu Arg Thr Trp Phe Ala
        115                 120                 125

Ala Asp Lys Thr Gly Phe His Met Leu His Thr Leu Phe Gln Thr Ser
    130                 135                 140

Leu Gln Phe Pro Gln Ile Gln Arg Phe Asp Glu His Phe Val Leu Asp
145                 150                 155                 160

Ile Leu Val Asp Asp Gly His Val Arg Gly Leu Val Ala Met Asn Met
                165                 170                 175

Met Glu Gly Thr Leu Val Gln Ile Arg Ala Asn Ala Val Val Met Ala
            180                 185                 190

Thr Gly Gly Ala Gly Arg Val Tyr Arg Tyr Asn Thr Asn Gly Gly Ile
        195                 200                 205

Val Thr Gly Asp Gly Met Gly Met Ala Leu Ser His Gly Val Pro Leu
    210                 215                 220
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Arg Asp Met Glu Phe Val Gln Tyr His Pro Thr Gly Leu Pro Gly Ser
225                 230                 235                 240

Gly Ile Leu Met Thr Glu Gly Cys Arg Gly Glu Gly Gly Ile Leu Val
                245                 250                 255

Asn Lys Asn Gly Tyr Arg Tyr Leu Gln Asp Tyr Gly Met Gly Pro Glu
            260                 265                 270

Thr Pro Leu Gly Glu Pro Lys Asn Lys Tyr Met Glu Leu Gly Pro Arg
        275                 280                 285

Asp Lys Val Ser Gln Ala Phe Trp His Glu Trp Arg Lys Gly Asn Thr
    290                 295                 300

Ile Ser Thr Pro Arg Gly Asp Val Val Tyr Leu Asp Leu Arg His Leu
305                 310                 315                 320

Gly Glu Lys Lys Leu His Glu Arg Leu Pro Phe Ile Cys Glu Leu Ala
                325                 330                 335

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

Pro Thr Ala His Tyr Thr Met Gly Gly Ile Glu Thr Asp Gln Asn Cys
        355                 360                 365

Glu Thr Arg Ile Lys Gly Leu Phe Ala Val Gly Glu Cys Ser Ser Val
    370                 375                 380

Gly Leu His Gly Ala Asn Arg Leu Gly Ser Asn Ser Leu Ala Glu Leu
385                 390                 395                 400

Val Val Phe Gly Arg Leu Ala Gly Glu Gln Ala Thr Glu Arg Ala Ala
                405                 410                 415

Thr Ala Gly Asn Gly Asn Glu Ala Ala Ile Glu Ala Gln Ala Ala Gly
            420                 425                 430

Val Glu Gln Arg Leu Lys Asp Leu Val Asn Gln Asp Gly Gly Glu Asn
        435                 440                 445

Trp Ala Lys Ile Arg Asp Glu Met Gly Leu Ala Met Glu Glu Gly Cys
    450                 455                 460

Gly Ile Tyr Arg Thr Pro Glu Leu Met Gln Lys Thr Ile Asp Lys Leu
465                 470                 475                 480

Ala Glu Leu Gln Glu Arg Phe Lys Arg Val Arg Ile Thr Asp Thr Ser
                485                 490                 495

Ser Val Phe Asn Thr Asp Leu Leu Tyr Thr Ile Glu Leu Gly His Gly
            500                 505                 510

Leu Asn Val Ala Glu Cys Met Ala His Ser Ala Met Ala Arg Lys Glu
        515                 520                 525

Ser Arg Gly Ala His Gln Arg Leu Asp Glu Gly Cys Thr Glu Arg Asp
    530                 535                 540

Asp Val Asn Phe Leu Lys His Thr Leu Ala Phe Arg Asp Ala Asp Gly
545                 550                 555                 560

Thr Thr Arg Leu Glu Tyr Ser Asp Val Lys Ile Thr Thr Leu Pro Pro
                565                 570                 575

Ala Lys Arg Val Tyr Gly Gly Glu Ala Asp Ala Ala Asp Lys Ala Glu
            580                 585                 590

Ala Ala Asn Lys Lys Glu Lys Ala Asn Gly
        595                 600


<210> SEQ ID NO 23
<211> LENGTH: 735
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 23
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atggctgaga tgaaaaacct gaaaattgag gtggtgcgct ataacccgga agtcgatacc 60

gcaccgcata gcgcattcta tgaagtgcct tatgacgcaa ctacctcatt actggatgcg 120

ctgggctaca tcaaagacaa cctggcaccg gacctgagct accgctggtc ctgccgtatg 180

gcgatttgtg gttcctgcgg catgatggtt aacaacgtgc caaaactggc atgtaaaacc 240

ttcctgcgtg attacaccga cggtatgaag gttgaagcgt tagctaactt cccgattgaa 300

cgcgatctgg tggtcgatat gacccacttc atcgaaagtc tggaagcgat caaaccgtac 360

atcatcggca actcccgcac cgcggatcag ggtactaaca tccagacccc ggcgcagatg 420

gcgaagtatc accagttctc cggttgcatc aactgtggtt tgtgctacgc cgcgtgcccg 480

cagtttggcc tgaacccaga gttcatcggt ccggctgcca ttacgctggc gcatcgttat 540

aacgaagata gccgcgacca cggtaagaag gagcgtatgg cgcagttgaa cagccagaac 600

ggcgtatgga gctgtacttt cgtgggctac tgctccgaag tctgcccgaa acacgtcgat 660

ccggctgcgg ccattcagca gggcaaagta gaaagttcga aagactttct tatcgcgacc 720

ctgaaaccac gctaa 735

<210> SEQ ID NO 24
<211> LENGTH: 244
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 24

Met Ala Glu Met Lys Asn Leu Lys Ile Glu Val Val Arg Tyr Asn Pro
1 5 10 15

Glu Val Asp Thr Ala Pro His Ser Ala Phe Tyr Glu Val Pro Tyr Asp
20 25 30

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

Ala Pro Asp Leu Ser Tyr Arg Trp Ser Cys Arg Met Ala Ile Cys Gly
50 55 60

Ser Cys Gly Met Met Val Asn Asn Val Pro Lys Leu Ala Cys Lys Thr
65 70 75 80

Phe Leu Arg Asp Tyr Thr Asp Gly Met Lys Val Glu Ala Leu Ala Asn
85 90 95

Phe Pro Ile Glu Arg Asp Leu Val Val Asp Met Thr His Phe Ile Glu
100 105 110

Ser Leu Glu Ala Ile Lys Pro Tyr Ile Ile Gly Asn Ser Arg Thr Ala
115 120 125

Asp Gln Gly Thr Asn Ile Gln Thr Pro Ala Gln Met Ala Lys Tyr His
130 135 140

Gln Phe Ser Gly Cys Ile Asn Cys Gly Leu Cys Tyr Ala Ala Cys Pro
145 150 155 160

Gln Phe Gly Leu Asn Pro Glu Phe Ile Gly Pro Ala Ala Ile Thr Leu
165 170 175

Ala His Arg Tyr Asn Glu Asp Ser Arg Asp His Gly Lys Lys Glu Arg
180 185 190

Met Ala Gln Leu Asn Ser Gln Asn Gly Val Trp Ser Cys Thr Phe Val
195 200 205

Gly Tyr Cys Ser Glu Val Cys Pro Lys His Val Asp Pro Ala Ala Ala
210 215 220

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

Leu Lys Pro Arg

<210> SEQ ID NO 25
<211> LENGTH: 396
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 25

atgacgacta aacgtaaacc gtatgtacgg ccaatgacgt ccacctggtg gaaaaaattg 60

ccgttttatc gcttttacat gctgcgcgaa ggcacggcgg ttccggctgt gtggttcagc 120

attgaactga ttttcgggct gtttgccctg aaaaatggcc cggaagcctg ggcgggattc 180

gtcgactttt tacaaaaccc ggttatcgtg atcattaacc tgatcactct ggcggcagct 240

ctgctgcaca ccaaaacctg gtttgaactg gcaccgaaag cggccaatat cattgtaaaa 300

gacgaaaaaa tgggaccaga gccaattatc aaaagtctct gggcggtaac tgtggttgcc 360

accatcgtaa tcctgtttgt tgccctgtac tggtaa 396

<210> SEQ ID NO 26
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 26

Met Thr Thr Lys Arg Lys Pro Tyr Val Arg Pro Met Thr Ser Thr Trp
1 5 10 15

Trp Lys Lys Leu Pro Phe Tyr Arg Phe Tyr Met Leu Arg Glu Gly Thr
20 25 30

Ala Val Pro Ala Val Trp Phe Ser Ile Glu Leu Ile Phe Gly Leu Phe
35 40 45

Ala Leu Lys Asn Gly Pro Glu Ala Trp Ala Gly Phe Val Asp Phe Leu
50 55 60

Gln Asn Pro Val Ile Val Ile Ile Asn Leu Ile Thr Leu Ala Ala Ala
65 70 75 80

Leu Leu His Thr Lys Thr Trp Phe Glu Leu Ala Pro Lys Ala Ala Asn
85 90 95

Ile Ile Val Lys Asp Glu Lys Met Gly Pro Glu Pro Ile Ile Lys Ser
100 105 110

Leu Trp Ala Val Thr Val Val Ala Thr Ile Val Ile Leu Phe Val Ala
115 120 125

Leu Tyr Trp
130

<210> SEQ ID NO 27
<211> LENGTH: 360
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 27

atgattaatc caaatccaaa gcgttctgac gaaccggtat tctggggcct cttcggggcc 60

ggtggtatgt ggagcgccat cattgcgccg gtgatgatcc tgctggtggg tattctgctg 120

ccactggggt tgtttccggg tgatgcgctg agctacgagc gcgttctggc gttcgcgcag 180

agcttcattg gtcgcgtatt cctgttcctg atgatcgttc tgccgctgtg gtgtggttta 240

caccgtatgc accacgcgat gcacgatctg aaaatccacg tacctgcggg caaatgggtt 300

ttctacggtc tggctgctat cctgacagtt gtcacgctga ttggtgtcgt tacaatctaa 360

<210> SEQ ID NO 28
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 28

Met Ile Asn Pro Asn Pro Lys Arg Ser Asp Glu Pro Val Phe Trp Gly
1 5 10 15

Leu Phe Gly Ala Gly Gly Met Trp Ser Ala Ile Ile Ala Pro Val Met
20 25 30

Ile Leu Leu Val Gly Ile Leu Leu Pro Leu Gly Leu Phe Pro Gly Asp
35 40 45

Ala Leu Ser Tyr Glu Arg Val Leu Ala Phe Ala Gln Ser Phe Ile Gly
50 55 60

Arg Val Phe Leu Phe Leu Met Ile Val Leu Pro Leu Trp Cys Gly Leu
65 70 75 80

His Arg Met His His Ala Met His Asp Leu Lys Ile His Val Pro Ala
85 90 95

Gly Lys Trp Val Phe Tyr Gly Leu Ala Ala Ile Leu Thr Val Val Thr
100 105 110

Leu Ile Gly Val Val Thr Ile
115

<210> SEQ ID NO 29
<211> LENGTH: 1812
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 29

atgaatccac aattgttacg cgtaacaaat cgaatcattg aacgttcgcg cgagactcgc 60

tctgcttatc tcgcccggat agaacaagcg aaaacttcga ccgttcatcg ttcgcagttg 120

gcatgcggta acctggcaca cggtttcgct gcctgccagc cagaagacaa agcctctttg 180

aaaagcatgt tgcgtaacaa tatcgccatc atcacctcct ataacgacat gctctccgcg 240

caccagcctt atgaacacta tccagaaatc attcgtaaag ccctgcatga agcgaatgcg 300

gttggtcagg ttgcgggcgg tgttccggcg atgtgtgatg gtgtcaccca ggggcaggat 360

ggaatggaat tgtcgctgct aagccgcgaa gtgatagcga tgtctgcggc ggtggggctg 420

tcccataaca tgtttgatgg tgctctgttc ctcggtgtgt gcgacaagat tgtcccgggt 480

ctgacgatgg cagccctgtc gtttggtcat ttgcctgcgg tgtttgtgcc gtctggaccg 540

atggcaagcg gtttgccaaa taaagaaaaa gtgcgtattc gccagcttta tgccgaaggt 600

aaagtggacc gcatggcctt actggagtca gaagccgcgt cttaccatgc gccgggaaca 660

tgtactttct acggtactgc caacaccaac cagatggtgg tggagtttat ggggatgcag 720

ttgccaggct cttcttttgt tcatccggat tctccgctgc gcgatgcttt gaccgccgca 780

gctgcgcgtc aggttacacg catgaccggt aatggtaatg aatggatgcc gatcggtaag 840

atgatcgatg agaaagtggt ggtgaacggt atcgttgcac tgctggcgac cggtggttcc 900

actaaccaca ccatgcacct ggtggcgatg gcgcgcgcgg ccggtattca gattaactgg 960

gatgacttct ctgacctttc tgatgttgta ccgctgatgg cacgtctcta cccgaacggt 1020

ccggccgata ttaaccactt ccaggcggca ggtggcgtac cggttctggt gcgtgaactg 1080

ctcaaagcag gcctgctgca tgaagatgtc aatacggtgg caggttttgg tctgtctcgt 1140

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tataccttg aaccatggct gaataatggt gaactggact ggcgggaagg ggcggaaaaa 1200

tcactcgaca gcaatgtgat cgcttccttc gaacaacctt tctctcatca tggtgggaca 1260

aaagtgttaa gcggtaacct gggccgtgcg gttatgaaaa cctctgccgt gccggttgag 1320

aaccaggtga ttgaagcgcc agcggttgtt tttgaaagcc agcatgacgt tatgccggcc 1380

tttgaagcgg gtttgctgga ccgcgattgt gtcgttgttg tccgtcatca ggggccaaaa 1440

gcgaacggaa tgccagaatt acataaactc atgccgccac ttggtgtatt attggaccgg 1500

tgtttcaaaa ttgcgttagt taccgatgga cgactctccg gcgcttcagg taaagtgccg 1560

tcagctatcc acgtaacacc agaagcctac gatggcgggc tgctggcaaa agtgcgcgac 1620

ggggacatca ttcgtgtgaa tggacagaca ggcgaactga cgctgctggt agacgaagcg 1680

gaactggctg ctcgcgaacc gcacattcct gacctgagcg cgtcacgcgt gggaacagga 1740

cgtgaattat tcagcgcctt gcgtgaaaaa ctgtccggtg ccgaacaggg cgcaacctgt 1800

atcacttttt aa 1812

<210> SEQ ID NO 30
<211> LENGTH: 603
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 30

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

Arg Glu Thr Arg Ser Ala Tyr Leu Ala Arg Ile Glu Gln Ala Lys Thr
20 25 30

Ser Thr Val His Arg Ser Gln Leu Ala Cys Gly Asn Leu Ala His Gly
35 40 45

Phe Ala Ala Cys Gln Pro Glu Asp Lys Ala Ser Leu Lys Ser Met Leu
50 55 60

Arg Asn Asn Ile Ala Ile Ile Thr Ser Tyr Asn Asp Met Leu Ser Ala
65 70 75 80

His Gln Pro Tyr Glu His Tyr Pro Glu Ile Ile Arg Lys Ala Leu His
85 90 95

Glu Ala Asn Ala Val Gly Gln Val Ala Gly Gly Val Pro Ala Met Cys
100 105 110

Asp Gly Val Thr Gln Gly Gln Asp Gly Met Glu Leu Ser Leu Leu Ser
115 120 125

Arg Glu Val Ile Ala Met Ser Ala Ala Val Gly Leu Ser His Asn Met
130 135 140

Phe Asp Gly Ala Leu Phe Leu Gly Val Cys Asp Lys Ile Val Pro Gly
145 150 155 160

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

Pro Ser Gly Pro Met Ala Ser Gly Leu Pro Asn Lys Glu Lys Val Arg
180 185 190

Ile Arg Gln Leu Tyr Ala Glu Gly Lys Val Asp Arg Met Ala Leu Leu
195 200 205

Glu Ser Glu Ala Ala Ser Tyr His Ala Pro Gly Thr Cys Thr Phe Tyr
210 215 220

Gly Thr Ala Asn Thr Asn Gln Met Val Val Glu Phe Met Gly Met Gln
225 230 235 240

Leu Pro Gly Ser Ser Phe Val His Pro Asp Ser Pro Leu Arg Asp Ala
245 250 255

```
Leu Thr Ala Ala Ala Ala Arg Gln Val Thr Arg Met Thr Gly Asn Gly
            260                 265                 270

Asn Glu Trp Met Pro Ile Gly Lys Met Ile Asp Glu Lys Val Val Val
        275                 280                 285

Asn Gly Ile Val Ala Leu Leu Ala Thr Gly Gly Ser Thr Asn His Thr
    290                 295                 300

Met His Leu Val Ala Met Ala Arg Ala Ala Gly Ile Gln Ile Asn Trp
305                 310                 315                 320

Asp Asp Phe Ser Asp Leu Ser Asp Val Val Pro Leu Met Ala Arg Leu
                325                 330                 335

Tyr Pro Asn Gly Pro Ala Asp Ile Asn His Phe Gln Ala Ala Gly Gly
            340                 345                 350

Val Pro Val Leu Val Arg Glu Leu Leu Lys Ala Gly Leu Leu His Glu
        355                 360                 365

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

Pro Trp Leu Asn Asn Gly Glu Leu Asp Trp Arg Glu Gly Ala Glu Lys
385                 390                 395                 400

Ser Leu Asp Ser Asn Val Ile Ala Ser Phe Glu Gln Pro Phe Ser His
                405                 410                 415

His Gly Gly Thr Lys Val Leu Ser Gly Asn Leu Gly Arg Ala Val Met
            420                 425                 430

Lys Thr Ser Ala Val Pro Val Glu Asn Gln Val Ile Glu Ala Pro Ala
        435                 440                 445

Val Val Phe Glu Ser Gln His Asp Val Met Pro Ala Phe Glu Ala Gly
    450                 455                 460

Leu Leu Asp Arg Asp Cys Val Val Val Val Arg His Gln Gly Pro Lys
465                 470                 475                 480

Ala Asn Gly Met Pro Glu Leu His Lys Leu Met Pro Pro Leu Gly Val
                485                 490                 495

Leu Leu Asp Arg Cys Phe Lys Ile Ala Leu Val Thr Asp Gly Arg Leu
            500                 505                 510

Ser Gly Ala Ser Gly Lys Val Pro Ser Ala Ile His Val Thr Pro Glu
        515                 520                 525

Ala Tyr Asp Gly Gly Leu Leu Ala Lys Val Arg Asp Gly Asp Ile Ile
    530                 535                 540

Arg Val Asn Gly Gln Thr Gly Glu Leu Thr Leu Leu Val Asp Glu Ala
545                 550                 555                 560

Glu Leu Ala Ala Arg Glu Pro His Ile Pro Asp Leu Ser Ala Ser Arg
                565                 570                 575

Val Gly Thr Gly Arg Glu Leu Phe Ser Ala Leu Arg Glu Lys Leu Ser
            580                 585                 590

Gly Ala Glu Gln Gly Ala Thr Cys Ile Thr Phe
        595                 600
```

<210> SEQ ID NO 31
<211> LENGTH: 642
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 31

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atgaaaaact ggaaaacaag tgcagaatca atcctgacca ccggcccggt tgtaccggtt     60

atcgtggtaa aaaaactgga acacgcggtg ccgatggcaa aagcgttggt tgctggtggg    120
```

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gtgcgcgttc tggaagtgac tctgcgtacc gagtgtgcag ttgacgctat ccgtgctatc 180

gccaaagaag tgcctgaagc gattgtgggt gccggtacgg tgctgaatcc acagcagctg 240

gcagaagtca ctgaagcggg tgcacagttc gcaattagcc cgggtctgac cgagccgctg 300

ctgaaagctg ctaccgaagg gactattcct ctgattccgg ggatcagcac tgtttccgaa 360

ctgatgctgg gtatggacta cggtttgaaa gagttcaaat tcttcccggc tgaagctaac 420

ggcggcgtga aagccctgca ggcgatcgcg ggtccgttct cccaggtccg tttctgcccg 480

acgggtggta tttctccggc taactaccgt gactacctgg cgctgaaaag cgtgctgtgc 540

atcggtggtt cctggctggt tccggcagat gcgctggaag cgggcgatta cgaccgcatt 600

actaagctgg cgcgtgaagc tgtagaaggc gctaagctgt aa 642

<210> SEQ ID NO 32
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 32

Met Lys Asn Trp Lys Thr Ser Ala Glu Ser Ile Leu Thr Thr Gly Pro
1 5 10 15

Val Val Pro Val Ile Val Val Lys Lys Leu Glu His Ala Val Pro Met
20 25 30

Ala Lys Ala Leu Val Ala Gly Gly Val Arg Val Leu Glu Val Thr Leu
35 40 45

Arg Thr Glu Cys Ala Val Asp Ala Ile Arg Ala Ile Ala Lys Glu Val
50 55 60

Pro Glu Ala Ile Val Gly Ala Gly Thr Val Leu Asn Pro Gln Gln Leu
65 70 75 80

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

Thr Glu Pro Leu Leu Lys Ala Ala Thr Glu Gly Thr Ile Pro Leu Ile
100 105 110

Pro Gly Ile Ser Thr Val Ser Glu Leu Met Leu Gly Met Asp Tyr Gly
115 120 125

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

Ala Leu Gln Ala Ile Ala Gly Pro Phe Ser Gln Val Arg Phe Cys Pro
145 150 155 160

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

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

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

Glu Gly Ala Lys Leu
210

<210> SEQ ID NO 33
<211> LENGTH: 1707
<212> TYPE: DNA
<213> ORGANISM: Zymomonas mobilis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (622)..(622)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 33

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atgagttata ctgtcggtac ctatttagcg gagcggcttg tccagattgg tctcaagcat 60

cacttcgcag tcgcgggcga ctacaacctc gtccttcttg acaacctgct tttgaacaaa 120

aacatggagc aggtttattg ctgtaacgaa ctgaactgcg gtttcagtgc agaaggttat 180

gctcgtgcca aaggcgcagc agcagccgtc gttacctaca gcgtcggtgc gctttccgca 240

tttgatgcta tcggtggcgc ctatgcagaa aaccttccgg ttatcctgat ctccggtgct 300

ccgaacaaca atgaccacgc tgctggtcac gtgttgcatc acgctcttgg caaaaccgac 360

tatcactatc agttggaaat ggccaagaac atcacggccg ccgctgaagc gatttatacc 420

ccggaagaag ctccggctaa aatcgatcac gtgattaaaa ctgctcttcg tgagaagaag 480

ccggtttatc tcgaaatcgc ttgcaacatt gcttccatgc cctgcgccgc tcctggaccg 540

gcaagcgcat tgttcaatga cgaagccagc gacgaagctt ctttgaatgc agcggttgaa 600

gaaaccctga aattcatcgc cnaccgcgac aaagttgccg tcctcgtcgg cagcaagctg 660

cgcgcagctg gtgctgaaga agctgctgtc aaatttgctg atgctcttgg tggcgcagtt 720

gctaccatgg ctgctgcaaa aagcttcttc ccagaagaaa acccgcatta catcggtacc 780

tcatggggtg aagtcagcta tccgggcgtt gaaaagacga tgaaagaagc cgatgcggtt 840

atcgctctgg ctcctgtctt taacgactac tccaccactg gttggacgga tattcctgat 900

cctaagaaac tggttctcgc tgaaccgcgt tctgtcgtcg ttaacggcat tcgcttcccc 960

agcgtccatc tgaaagacta tctgacccgt ttggctcaga aagtttccaa gaaaaccggt 1020

gctttggact tcttcaaatc cctcaatgca ggtgaactga agaaagccgc tccggctgat 1080

ccgagtgctc cgttggtcaa cgcagaaatc gcccgtcagg tcgaagctct tctgaccccg 1140

aacacgacgg ttattgctga aaccggtgac tcttggttca atgctcagcg catgaagctc 1200

ccgaacggtg ctcgcgttga atatgaaatg cagtggggtc acattggttg gtccgttcct 1260

gccgccttcg gttatgccgt cggtgctccg gaacgtcgca acatcctcat ggttggtgat 1320

ggttccttcc agctgacggc tcaggaagtc gctcagatgg ttcgcctgaa actgccggtt 1380

atcatcttct tgatcaataa ctatggttac accatcgaag ttatgatcca tgatggtccg 1440

tacaacaaca tcaagaactg ggattatgcc ggtctgatgg aagtgttcaa cggtaacggt 1500

ggttatgaca gcggtgctgg taaaggcctg aaggctaaaa ccggtggcga actggcagaa 1560

gctatcaagg ttgctctggc aaacaccgac ggcccaaccc tgatcgaatg cttcatcggt 1620

cgtgaagact gcactgaaga attggtcaaa tggggtaagc gcgttgctgc cgccaacagc 1680

cgtaagcctg ttaacaagct cctctag 1707

<210> SEQ ID NO 34
<211> LENGTH: 568
<212> TYPE: PRT
<213> ORGANISM: Zymomonas mobilis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (208)..(208)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 34

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

Gly Leu Lys His His Phe Ala Val Ala Gly Asp Tyr Asn Leu Val Leu
20 25 30

Leu Asp Asn Leu Leu Leu Asn Lys Asn Met Glu Gln Val Tyr Cys Cys
35 40 45

-continued

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Asn Glu Leu Asn Cys Gly Phe Ser Ala Glu Gly Tyr Ala Arg Ala Lys
    50                  55                  60

Gly Ala Ala Ala Ala Val Val Thr Tyr Ser Val Gly Ala Leu Ser Ala
65                  70                  75                  80

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

Ile Ser Gly Ala Pro Asn Asn Asn Asp His Ala Ala Gly His Val Leu
            100                 105                 110

His His Ala Leu Gly Lys Thr Asp Tyr His Tyr Gln Leu Glu Met Ala
        115                 120                 125

Lys Asn Ile Thr Ala Ala Ala Glu Ala Ile Tyr Thr Pro Glu Glu Ala
    130                 135                 140

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

Pro Val Tyr Leu Glu Ile Ala Cys Asn Ile Ala Ser Met Pro Cys Ala
                165                 170                 175

Ala Pro Gly Pro Ala Ser Ala Leu Phe Asn Asp Glu Ala Ser Asp Glu
            180                 185                 190

Ala Ser Leu Asn Ala Ala Val Glu Glu Thr Leu Lys Phe Ile Ala Xaa
        195                 200                 205

Arg Asp Lys Val Ala Val Leu Val Gly Ser Lys Leu Arg Ala Ala Gly
    210                 215                 220

Ala Glu Glu Ala Ala Val Lys Phe Ala Asp Ala Leu Gly Gly Ala Val
225                 230                 235                 240

Ala Thr Met Ala Ala Ala Lys Ser Phe Phe Pro Glu Glu Asn Pro His
                245                 250                 255

Tyr Ile Gly Thr Ser Trp Gly Glu Val Ser Tyr Pro Gly Val Glu Lys
            260                 265                 270

Thr Met Lys Glu Ala Asp Ala Val Ile Ala Leu Ala Pro Val Phe Asn
        275                 280                 285

Asp Tyr Ser Thr Thr Gly Trp Thr Asp Ile Pro Asp Pro Lys Lys Leu
    290                 295                 300

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

Ser Val His Leu Lys Asp Tyr Leu Thr Arg Leu Ala Gln Lys Val Ser
                325                 330                 335

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

Leu Lys Lys Ala Ala Pro Ala Asp Pro Ser Ala Pro Leu Val Asn Ala
        355                 360                 365

Glu Ile Ala Arg Gln Val Glu Ala Leu Leu Thr Pro Asn Thr Thr Val
    370                 375                 380

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

Pro Asn Gly Ala Arg Val Glu Tyr Glu Met Gln Trp Gly His Ile Gly
                405                 410                 415

Trp Ser Val Pro Ala Ala Phe Gly Tyr Ala Val Gly Ala Pro Glu Arg
            420                 425                 430

Arg Asn Ile Leu Met Val Gly Asp Gly Ser Phe Gln Leu Thr Ala Gln
        435                 440                 445

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

Tyr Asn Asn Ile Lys Asn Trp Asp Tyr Ala Gly Leu Met Glu Val Phe
                485                 490                 495

Asn Gly Asn Gly Gly Tyr Asp Ser Gly Ala Gly Lys Gly Leu Lys Ala
            500                 505                 510

Lys Thr Gly Gly Glu Leu Ala Glu Ala Ile Lys Val Ala Leu Ala Asn
        515                 520                 525

Thr Asp Gly Pro Thr Leu Ile Glu Cys Phe Ile Gly Arg Glu Asp Cys
    530                 535                 540

Thr Glu Glu Leu Val Lys Trp Gly Lys Arg Val Ala Ala Ala Asn Ser
545                 550                 555                 560

Arg Lys Pro Val Asn Lys Leu Leu
                565
```

<210> SEQ ID NO 35
<211> LENGTH: 1152
<212> TYPE: DNA
<213> ORGANISM: Zymomonas mobilis

<400> SEQUENCE: 35

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atggcttctt caacttttta tattcctttc gtcaacgaaa tgggcgaagg ttcgcttgaa     60

aaagcaatca aggatcttaa cggcagcggc tttaaaaatg cgctgatcgt ttctgatgct    120

ttcatgaaca aatccggtgt tgtgaagcag gttgctgacc tgttgaaagc acagggtatt    180

aattctgctg tttatgatgg cgttatgccg aacccgactg ttaccgcagt tctggaaggc    240

cttaagatcc tgaaggataa caattcagac ttcgtcatct ccctcggtgg tggttctccc    300

catgactgcg ccaaagccat cgctctggtc gcaaccaatg gtggtgaagt caaagactac    360

gaaggtatcg acaaatctaa gaaacctgcc ctgcctttga tgtcaatcaa cacgacggct    420

ggtacggctt ctgaaatgac gcgtttctgc atcatcactg atgaagtccg tcacgttaag    480

atggccattg ttgaccgtca cgttaccccg atggtttccg tcaacgatcc tctgttgatg    540

gttggtatgc caaaaggcct gaccgccgcc accggtatgg atgctctgac ccacgcattt    600

gaagcttatt cttcaacggc agctactccg atcaccgatg cttgcgcctt gaaggctgcg    660

tccatgatcg ctaagaatct gaagaccgct tgcgacaacg gtaaggatat gccagctcgt    720

gaagctatgg cttatgccca attcctcgct ggtatggcct tcaacaacgc ttcgcttggt    780

tatgtccatg ctatggctca ccagttgggc ggctactaca acctgccgca tggtgtctgc    840

aacgctgttc tgcttccgca tgttctggct tataacgcct ctgtcgttgc tggtcgtctg    900

aaagacgttg gtgttgctat gggtctcgat atcgccaatc tcggtgataa agaaggcgca    960

gaagccacca ttcaggctgt tcgcgatctg gctgcttcca ttggtattcc agcaaatctg   1020

accgagctgg gtgctaagaa agaagatgtg ccgcttcttg ctgaccacgc tctgaaagat   1080

gcttgtgctc tgaccaaccc gcgtcagggt gatcagaaag aagttgaaga actcttcctg   1140

agcgctttct aa                                                       1152
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<210> SEQ ID NO 36
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Zymomonas mobilis

<400> SEQUENCE: 36

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Met Ala Ser Ser Thr Phe Tyr Ile Pro Phe Val Asn Glu Met Gly Glu
1               5                   10                  15
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Gly Ser Leu Glu Lys Ala Ile Lys Asp Leu Asn Gly Ser Gly Phe Lys
            20                  25                  30

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

Lys Gln Val Ala Asp Leu Leu Lys Ala Gln Gly Ile Asn Ser Ala Val
    50                  55                  60

Tyr Asp Gly Val Met Pro Asn Pro Thr Val Thr Ala Val Leu Glu Gly
65                  70                  75                  80

Leu Lys Ile Leu Lys Asp Asn Asn Ser Asp Phe Val Ile Ser Leu Gly
                85                  90                  95

Gly Gly Ser Pro His Asp Cys Ala Lys Ala Ile Ala Leu Val Ala Thr
            100                 105                 110

Asn Gly Gly Glu Val Lys Asp Tyr Glu Gly Ile Asp Lys Ser Lys Lys
        115                 120                 125

Pro Ala Leu Pro Leu Met Ser Ile Asn Thr Thr Ala Gly Thr Ala Ser
    130                 135                 140

Glu Met Thr Arg Phe Cys Ile Ile Thr Asp Glu Val Arg His Val Lys
145                 150                 155                 160

Met Ala Ile Val Asp Arg His Val Thr Pro Met Val Ser Val Asn Asp
                165                 170                 175

Pro Leu Leu Met Val Gly Met Pro Lys Gly Leu Thr Ala Ala Thr Gly
            180                 185                 190

Met Asp Ala Leu Thr His Ala Phe Glu Ala Tyr Ser Ser Thr Ala Ala
        195                 200                 205

Thr Pro Ile Thr Asp Ala Cys Ala Leu Lys Ala Ala Ser Met Ile Ala
    210                 215                 220

Lys Asn Leu Lys Thr Ala Cys Asp Asn Gly Lys Asp Met Pro Ala Arg
225                 230                 235                 240

Glu Ala Met Ala Tyr Ala Gln Phe Leu Ala Gly Met Ala Phe Asn Asn
                245                 250                 255

Ala Ser Leu Gly Tyr Val His Ala Met Ala His Gln Leu Gly Gly Tyr
            260                 265                 270

Tyr Asn Leu Pro His Gly Val Cys Asn Ala Val Leu Leu Pro His Val
        275                 280                 285

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

Val Ala Met Gly Leu Asp Ile Ala Asn Leu Gly Asp Lys Glu Gly Ala
305                 310                 315                 320

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

Pro Ala Asn Leu Thr Glu Leu Gly Ala Lys Lys Glu Asp Val Pro Leu
            340                 345                 350

Leu Ala Asp His Ala Leu Lys Asp Ala Cys Ala Leu Thr Asn Pro Arg
        355                 360                 365

Gln Gly Asp Gln Lys Glu Val Glu Glu Leu Phe Leu Ser Ala Phe
    370                 375                 380
```

We claim:

1. A recombinant microorganism comprising:

a genetic modification that reduces or ablates the activity of HPr of *E. coli* or an ortholog thereof and FPr of *E. coli* or an ortholog thereof; and

at least one of:

a genetic modification that reduces or ablates the activity of a pyruvate formate lyase;

a genetic modification that reduces or ablates the activity of a lactate dehydrogenase;

a genetic modification that reduces or ablates the activity of a fumarate reductase;

a recombinant pyruvate decarboxylase gene; and

a recombinant alcohol dehydrogenase gene.

2. The recombinant microorganism of claim 1 wherein the microorganism is a bacterium.

3. The recombinant microorganism of claim 1 comprising a genetic modification that reduces or ablates the activity of a pyruvate formate lyase.

4. The recombinant microorganism of claim 1 comprising a genetic modification that reduces or ablates the activity of a lactate dehydrogenase.

5. The recombinant microorganism of claim 1 comprising a genetic modification that reduces or ablates the activity of a fumarate reductase.

6. The recombinant microorganism of claim 1 comprising a recombinant pyruvate decarboxylase gene and a recombinant alcohol dehydrogenase gene.

7. The recombinant microorganism of claim 1 comprising:

a genetic modification that reduces or ablates the activity of a pyruvate formate lyase;

a genetic modification that reduces or ablates the activity of a lactate dehydrogenase;

a genetic modification that reduces or ablates the activity of a fumarate reductase;

a recombinant pyruvate decarboxylase gene; and

a recombinant alcohol dehydrogenase gene.

8. The recombinant microorganism of claim 1 further comprising a genetic modification that reduces or ablates the activity of a PTS protein selected from the group consisting of an EI and an EII^{Glc}.

9. The recombinant microorganism of claim 1 further comprising a genetic modification that reduces or ablates the activity of a PTS protein selected from the group consisting of an EI and an EII^{Glc}, and comprising:

a genetic modification that reduces or ablates the activity of a pyruvate formate lyase;

a genetic modification that reduces or ablates the activity of a lactate dehydrogenase;

a genetic modification that reduces or ablates the activity of a fumarate reductase;

a recombinant pyruvate decarboxylase gene; and

a recombinant alcohol dehydrogenase gene.

10. A method of consuming a carbohydrate comprising culturing the recombinant microorganism of claim 1 in a medium comprising the carbohydrate, wherein the microorganism consumes the carbohydrate during the culturing.

11. The method of claim 10 wherein the medium comprises glucose and xylose.

12. The method of claim 11 wherein the microorganism consumes at least about 10% of an initial amount of the xylose in the medium during the time the microorganism consumes about 20% of an initial amount of the glucose in the medium.

13. The method of claim 10 wherein the medium comprises a biomass hydrolysate.

14. The method of claim 13 wherein the biomass hydrolysate is an enzymatic hydrolysate, an acid hydrolysate, or a hydrolysate of an ionic liquid.

15. The method of claim 10 wherein the microorganism is adapted to growth in a first medium comprising a component selected from the group consisting of glucose, xylose, and ethanol prior to culturing the microorganism in the medium.

16. The method of claim 10 wherein the culturing produces at least about 300 mM ethanol.

17. The recombinant microorganism of claim 1 comprising at least one of a recombinant pyruvate decarboxylase gene and a recombinant alcohol dehydrogenase gene.

18. The recombinant microorganism of claim 1, wherein each genetic modification is independently selected from the group consisting of a genetic mutation in a coding sequence, a genetic mutation in a sequence controlling transcription of a coding sequence, a genetic mutation in a sequence controlling translation of a coding sequence, a non-native nucleic acid configured to express a ribozyme that targets an mRNA of a coding sequence, and a non-native nucleic acid configured to express an antisense sequence that targets an mRNA of a coding sequence.

19. The recombinant microorganism of claim 1, wherein each genetic modification is independently selected from the group consisting of a genetic mutation in a coding sequence that reduces or ablates expression of a gene product of the coding sequence, a genetic mutation in a sequence controlling transcription of a coding sequence that reduces or ablates expression of a gene product of the coding sequence, a genetic mutation in a sequence controlling translation of a coding sequence that reduces or ablates expression of a gene product of the coding sequence, a genetic mutation in a coding sequence that reduces or ablates activity of a gene product expressed from the coding sequence, a non-native nucleic acid configured to express a ribozyme that targets an mRNA of a coding sequence, and a non-native nucleic acid configured to express an antisense sequence that targets an mRNA of a coding sequence.

20. The recombinant microorganism of claim 1, wherein each genetic modification is independently selected from the group consisting of a substitution in a coding sequence, a substitution in a sequence controlling transcription of a coding sequence, a substitution in sequence controlling translation of a coding sequence, an insertion in a coding sequence, an insertion in a sequence controlling transcription of a coding sequence, an insertion in a sequence controlling translation of a coding sequence, a partial deletion of a coding sequence, a partial deletion of a sequence controlling transcription of a coding sequence, a partial deletion of a sequence controlling translation of a coding sequence, a complete deletion of a coding sequence, a complete deletion of a sequence controlling transcription of a coding sequence, a complete deletion of a sequence controlling translation of a coding sequence, a non-native nucleic acid configured to express a ribozyme that targets an mRNA of a coding sequence, and a non-native nucleic acid configured to express an antisense sequence that targets an mRNA of a coding sequence.

* * * * *