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(54) Title: OVER-EXPRESSION OF HADH-DEPENDENT OXIDOREDUCTASE (FUCO) FOR INCREASING FURFURAL OR 5-HYDROXYMETHYLFURFURAL TOLERANCE

(57) Abstract: The subject invention pertains to the discovery that the NADH-dependent propanediol oxidoreductase (FucO) can reduce furfural. This allows for a new approach to improve furfural tolerance in bacterial and/or yeast cells used to produce desired products. Thus, novel biocatalysts (bacterial, fungal or yeast cells) exhibiting increased tolerance to furfural and 5-hydroxymethylfurfural (5-HMF) are provided as are methods of making and using such biocatalysts for the production of a desired product.

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DESCRIPTION

OVER-EXPRESSION OF NADH-DEPENDENT OXIDOREDUCTASE (FUCO) FOR INCREASING FURFURAL OR 5-HYDROXYMETHYLFURFURAL TOLERANCE

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S Provisional Patent Application Serial Number 61/470,642, filed April 1, 2011, the disclosure of which is hereby incorporated by reference in its entirety, including all figures, tables and nucleic acid sequences.

This invention was made with government support under DE-FG36-08GO88142 awarded by Department of Energy. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Carbohydrate components of woody biomass (cellulose and hemicellulose) represent an abundant potential source of sugars for microbial conversion into renewable fuels, plastics, and other chemicals (7, 18, 35). However, cost-effective depolymerization of this complex material to produce fermentable sugar streams remains a major challenge (3, 35). Pretreatment processes such as dilute mineral acids at elevated temperature and pressures open the structure of woody biomass to increase the effectiveness of cellulase enzymes, and hydrolyze the pentose polymers of hemicellulose into monomers. Unwanted side reactions from this pretreatment also produce a mixture of compounds (furans, acetate, soluble products from lignin, and others) that inhibit growth and retard fermentation (1, 18, 31). Most inhibitors can be removed or neutralized by separating the solubilized sugars from the cellulose-enriched fiber using counter-current washing followed by over-liming (25, 26). However, these added process steps would also add cost to renewable products. By developing robust biocatalysts that are resistant to side products from pretreatment it should be possible to design a simpler process (13, 14).

Furfural, the dehydration product of xylose, is of particular importance as a fermentation inhibitor in hemicellulose hydrolysates (1, 31). Furfural concentrations in hemicellulose hydrolysates have been correlated with toxicity (39). The addition of furfural to over-limed hemicellulose hydrolysates has been shown to restore toxicity (25, 26). In model studies with various hydrolysate inhibitors, furfural was unique in potentiating the toxicity of other compounds (39). Furan alcohols (reduced products) are less toxic than the respective aldehydes (38, 39). Several genes encoding oxidoreductases that reduce furfural and 5-hydroxymethylfurfural (5-HMF; dehydration product of hexose sugars) have been implicated in furan tolerance in *Saccharomyces cerevisiae* (2, 20, 22, 23) and in *E. coli* (28-30, 37).

Furfural-resistant mutants of ethanologenic *Escherichia coli* have been isolated and characterized (28, 29, 37). Resistance to low concentrations of furfural was found to result from the silencing of *yqhD*, an NADPH-dependent, furfural oxidoreductase that is induced by furfural (28, 29, 37). Although there are multiple NADPH-furfural reductases in *E. coli* and conversion of furfural to the less toxic alcohol would be generally regarded as beneficial, the unusually low K_m of YqhD for NADPH appears to compete with biosynthesis for NADPH (29). Metabolic routes for the anaerobic production of NADPH during xylose fermentation are quite limited (12, 16, 34). The metabolism of furfural by YqhD is proposed to inhibit growth and fermentation by depleting the pool of NADPH below that required for essential biosynthetic reactions (28, 29, 37). Sulfate assimilation was identified as a site that is particularly sensitive to NADPH limitation (28). Furan toxicity (furfural and 5-HMF) can be minimized by a variety of approaches that increased the availability of NADPH (Fig. 1) (28-30).

NADH is abundant during fermentation and represents a preferred reductant for furfural conversion to the less toxic alcohol, eliminating any burden on the NADPH pool. Our laboratory previously cloned the *E. coli fucO* gene (11), an NADH-dependent, L-1,2 propanediol reductase that is induced during fucose catabolism (8, 10).

BRIEF SUMMARY OF THE INVENTION

Furfural is an important fermentation inhibitor in hemicellulose sugar syrups derived from woody biomass. The metabolism of furfural by NADPH-dependent oxidoreductases such as YqhD (low K_m for NADPH) is proposed to inhibit the growth and fermentation of xylose in *Escherichia coli* by competing with biosynthesis for NADPH. The discovery that the NADH-dependent propanediol oxidoreductase (FucO) can reduce furfural has provided a new approach to improve furfural tolerance in bacterial, fungal and yeast cells used to produce desired products. Thus, novel biocatalysts (bacterial, fungal and yeast cells) exhibiting increased tolerance to furfural and 5-hydroxymethylfurfural (5-HMF) are provided as are methods of making and using such biocatalysts.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Role of cofactor metabolism in mediating furfural inhibition of growth. During xylose fermentation, small amounts of NADPH are produced for essential biosynthetic reactions. A partial list of genes encoding activities that could replenish NADPH is also shown. Furfural metabolism by NADPH-dependent oxidoreductases such as YqhD inhibits growth by depleting the NADPH pool (28, 29, 37). NADH-dependent fufural reductases such as FucO can increase furfural tolerance by reducing furfural to the less toxic furfuryl alcohol without depleting the NADPH pool.

Figures 2A-2D. Effect of *fucO* overexpression in LY180. Figure 2A. NADPH-dependent reduction of furfural and 5-HMF by cell-free extracts of LY180 containing vector alone (pTrc99A) and IPTG-induced *fucO* in LY180(pLOI4319). Figure 2B. NADH-dependent reduction of furfural and 5-HMF by cell-free extracts of LY180(pTrc99A) and IPTG-induced *fucO* in LY180(pLOI4319). In Figs 2A and 2B, open bars represent activity with furfural (10 mM) as a substrate. Gray bars represent activity with 5-HMF (10 mM). Figure 2C. In vivo furfural (10 mM) reduction by chloramphenicol-inhibited, non-growing cells (LY180, 0.88 mg cell dry weight ml^{-1}) containing vector alone (pTrc99A) or 0.1 mM IPTG-induced *fucO* in LY180(pLOI4319). Figure 2D. Growth inhibition of LY180(pTrc99A) with vector alone (Δ, 0 mM IPTG) or pLOI4319 (○, 0 mM IPTG; ●, pLOI4319 induced with 0.1 mM IPTG).

Figures 3A-3C. Effects of media supplements and *fucO* expression on furfural tolerance. Strains LY180(pTrc99A) and LY180(pLOI4319) were grown for 48 h in tube cultures containing AM1 medium, 15 mM furfural (A and B) or 10 mM furfural (C). IPTG (0.1 mM) was also included with LY180(pLOI4319) to induce *fucO*. Bars indicate presence (solid) or absence (open) of supplement. Figure 3A. Yeast extract (1 g liter^{-1}). Figure 3B. Cysteine (0.1 mM). Figure 3C. AM1-glucose (50 g liter^{-1}) replacing xylose.

Figures 4A-4C. Effect of *fucO* expression on furfural tolerance during ethanol production from xylose. Batch fermentations were conducted in pH-controlled fermentation vessels in the absence and presence of furfural (15 mM). Expression of *fucO* from pLOI4319 (0.1 mM IPTG) was compared to vector controls (pTrc99A) using host strains LY180 and EM322. LY180 strains were unable to grow under these conditions but continued to metabolize furfural. Controls were included without furfural (open symbols and dotted lines). Figure 4A. Cell Mass. Figure 4B. Ethanol. Figure 4C. Furfural.

Figures 5A-5F. Effect of *fucO* expression on furfural tolerance during D-lactate production from xylose. Batch fermentations were conducted in pH-controlled fermentation vessels in the absence and presence of furfural. Expression of *fucO* from pLOI4319 (0.1 mM IPTG) was compared to vector controls (pTrc99A) using XW068 as the host. Controls were included without furfural (open symbols and dotted lines). Figure 5A. Cell mass (10 mM furfural). Figure 5B. D-lactate (10 mM furfural). Figure 5C. Furfural (10 mM furfural). Figure 5D. Cell mass (15 mM furfural). Figure 5E. D-lactate (15 mM furfural). Figure 5F. Furfural (15 mM furfural).

Figure 6. Chromosomal integration *adhE:: fucO* increased furfural resistance for succinate producing *E. coli* strains. The strains XW055, XW056, XW057 and XW081 were grown in AM1 medium, 50 g liter^{-1} xylose, 100 mM MOPS (pH 7.0), 50 mM $KHCO_3$ in the presence of 10 mM furfural for 48 h at 37°C. The genetic features about *yqhD* and *fucO* integration are indicated below the graphs.

DETAILED DISCLOSURE OF THE INVENTION

The invention provides organisms for production of renewable fuels and other chemicals. Particularly, the invention provides bacteria, fungi and yeast that can grow and produce renewable fuels and other chemicals in the presence of increased furfural. The invention provides for an isolated or recombinant cell (bacterial, yeast or fungal cell) having increased expression of at least one NADH-dependent oxidoreductase (for example, the *FucO* gene) and that exhibits improved ability to reduce furfural and 5-HMF as compared to a reference cell (e.g., a reference bacterial, yeast or fungal cell). In various embodiments, the bacterial, fungal or yeast cell has increased furfural and 5-HMF tolerance as compared to a reference bacterial, fungal or yeast cell. The bacterial, fungal or yeast cell having increased furfural tolerance may be a wild-type bacterial, fungal or yeast cell that was selected for increased furfural and/or 5-HMF tolerance that is conferred by increased expression or activity of a NADH-dependent oxidoreductase (e.g., FucO). In various embodiments, the bacterial, fungal or yeast cell having increased furfural and/or 5-HMF tolerance can produce ethanol; lactic acid; succinic acid; malic acid; acetic acid; 1,3-propanediol; 2,3-propanediol; pyruvate; dicarboxylic acids; adipic acid; butanol; and amino acids, including aliphatic and aromatic amino acids.

Various publications have disclosed bacterial, fungal or yeast cells in which ethanol; lactic acid; succinic acid; malic acid; acetic acid; 1,3-propanediol; 2,3-propanediol; 1,4-

butanediol; 2,3-butanediol; butanol; pyruvate; dicarboxylic acids; adipic acid; and amino acids, including aliphatic and aromatic amino acids can be produced. Many of these microorganisms have been genetically manipulated (genetically engineered) in order to produce these desired products. Exemplary publications in this regard include U.S. Published Patent Applications US-2010/0184171A1 (directed to the production of malic acid and succinic acid), 2009/0148914A1 (directed to the production of acetic acid; 1,3-propanediol; 2,3-propanediol; pyruvate; dicarboxylic acids; adipic acid; and amino acids, including aliphatic and aromatic amino acids), 2007/0037265A1 (directed to the production of chirally pure D and L lactic acid) and PCT application PCT/US2010/029728 (published as WO2010/115067 and directed to the production of succinic acid). The teachings of each of these publications, with respect to the production of bacterial cells producing a desired product, is hereby incorporated by reference in its entirety.

In another aspect of the invention, bacterial, fungal or yeast cells disclosed herein demonstrate increased growth in the presence of furfural and/or 5-HMF as compared to a reference bacterial, fungal or yeast cell. In another embodiment, the bacterial, fungal or yeast cell has increased growth in the presence of furfural and/or 5-HMF at concentrations of about 5mM, 10mM, 15mM, 20 mM, 25mM, 30mM, 35mM, 40 mM or higher (or between about 5 mM and about 20 mM furfural and/or 5-HMF, about 15mM to about 30 mM furfural and/or 5-HMF, preferably about 15 mM furfural and/or 5 HMF).

Yet other aspects of the invention provide bacterial cells, fungal cells and yeast that demonstrate increased furfural and/or 5-HMF tolerance (have increased FucO activity), as compared to reference bacterial, fungal or yeast cells. Bacterial cells can be selected Gram negative bacteria or Gram positive bacteria. In this aspect of the invention, the Gram-negative bacterial cell can be selected from the group consisting of *Escherichia, Zymomonas, Acinetobacter, Gluconobacter, Geobacter, Shewanella, Salmonella, Enterobacter* and *Klebsiella.* Gram-positive bacteria can be selected from the group consisting of *Bacillus, Clostridium, Corynebacterial, Lactobacillus, Lactococcus, Oenococcus, Streptococcus* and *Eubacterial cells.* Various thermophilic bacterial cells, such as *Thermoanaerobes* (e.g., *Thermoanaerobacterium saccharolyticum*) can also be manipulated to increase furfural resistance and/or 5-HMF resistance. Other thermophilic microorganisms include, but are not limited to, *Bacillus spp., e.g., Bacillus coagulans* strains, *Bacillus licheniformis* strains, *Bacillus subtilis* strains, *Bacillus amyloliquifaciens* strains, *Bacillus megaterium* strains, *Bacillus macerans* strains, *Paenibacillus* spp. strains or *Geobacillus* spp. such as

Geobacillus stearothermophilus strains can be genetically modified. Other *Bacillus* strain can be obtained from culture collections such as ATCC (American Type Culture Collection) and modified to have increased FucO activity.

Other embodiments provide for a yeast cell or fungal cell having increased FucO activity. The yeast cell may be a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, or *Yarrowia* cell such as a *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis*, or *Yarrowia lipolytica* cell.

In other embodiments, the cell having increased FucO activity may be a fungal cell. "Fungi" as used herein includes the phyla *Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota, Oomycota* and all mitosporic fungi. A fungal cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (*Endomycetales*), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (*Blastomycetes*). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F. A., Passmore, S. M., and Davenport, R. R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980).

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision *Eumycota* and *Oomycota* (as defined by Hawksworth et al., 1995, supra). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

The filamentous fungal host cell may be an *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes*, or *Trichoderma* cell.

For example, the filamentous fungal host cell may be an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina,* *Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei,* or *Trichoderma viride* cell.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are described in EP 238023, Yelton et al., 1984, Proc. Natl. Acad. Sci. USA 81: 1470-1474, and Christensen et al., 1988, Bio/Technology 6: 1419-1422. Suitable methods for transforming *Fusarium* species are described by Malardier et al., 1989, Gene 78: 147-156, 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, J. Bacteriol. 153: 163; and Hinnen et al., 1978, Proc. Natl. Acad. Sci. USA 75: 1920.

In various embodiments within this aspect of the invention, the bacterial cells can be *Escherichia coli* or *Klebsiella oxytoca.* The invention provides for an isolated or recombinant bacterial cell, wherein FucO activity is increased as compared to a reference bacterial cell. In certain embodiments of this aspect of the invention, bacterial cells can also have YqhD activity decreased or inactivated.

The activity of FucO can be increased in a variety of ways. For example, FucO activity can be increased by expressing the *FucO* gene in a multicopy plasmid with a native promoter or any other promoter sequence which is known to increase gene expression.

Expression of FucO can also be increased by integrating additional copies of the *FucO* gene within the chromosome of a bacterial cell using transposons. Alternatively, the native promoter of the *FucO* gene can be replaced by other promoter elements known to enhance the level of gene expression in a bacterial cell. Other techniques for increasing FucO activity, for example mutation of the Fuco improve its kinetic properties or reduce its Km, can also be used to increase the activity of the oxidase. Similar techniques can be used for fungal and yeast cells.

Various other aspects of the invention provide methods of producing ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid or amino acids. In these aspects of the invention, known bacterial, fungal or yeast cells that produce ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, pyruvate, dicarboxylic acids, adipic acid or amino acids are manipulated in a manner that results in an increase in FucO activity for the bacterial, fungal or yeast cell (as compared to a reference bacterial, fungal or yeast cell). In various embodiments, the methods comprise culturing a bacterial, fungal or yeast cell producing a desired product (e.g., ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, pyruvate, dicarboxylic acids, adipic acid or amino acids) and having increased FucO activity, as compared to a reference cell, under conditions that allow for the production of the desired product. The desired product (e.g., ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, pyruvate, dicarboxylic acids, adipic acid or amino acids) can, optionally, be purified from the culture medium in which the bacterial, fungal or yeast cell was cultured. In various other embodiments, the bacterial, fungal or yeast cells can be cultured in the presence of a hemicellulose hydrolysate.

As used herein, “isolated” refers to bacterial, fungal or yeast cells partially or completely free from contamination by other bacteria. An isolated bacterial, fungal or yeast cell (bacterial, fungal or yeast cell) can exist in the presence of a small fraction of other bacteria which do not interfere with the properties and function of the isolated bacterial, fungal or yeast cell (e.g., a bacterial, fungal or yeast cell having increased FucO activity). An isolated bacterial, fungal or yeast cell will generally be at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 99% pure. Preferably, an isolated bacterial, fungal or yeast cell according to the invention will be at least 98% or at least 99% pure.

A bacterial, fungal or yeast cell may be a recombinant, non-recombinant or mutant isolated from nature, provided that the bacterial, fungal or yeast cell exhibits higher levels of FucO activity as compared to a reference strain. A non-recombinant bacterial, fungal or yeast cell includes a bacterial, fungal or yeast cell that does not contain heterologous polynucleotide sequences, and is suitable for further modification as disclosed herein, including genetic manipulation for the introduction of heterologous polynucleotide sequences. The term is intended to include progeny of the cell originally transfected. A "recombinant cell" is a bacterial, fungal or yeast cell that contains a heterologous polynucleotide sequence, or that has been treated such that a native polynucleotide sequence has been mutated or deleted. A "mutant" bacterial, fungal or yeast cell is a cell that is not identical to a reference bacterial, fungal or yeast cell, as defined herein below.

A wild-type bacterial, fungal or yeast cell is the typical form of an organism or strain, for example a bacterial cell, as it occurs in nature, in the absence of mutations. Wild-type refers to the most common phenotype in the natural population. "Parental bacterial, fungal or yeast strain", "parental bacterial strain", "parental fungal strain" or "parental yeast strain" is the standard of reference for the genotype and phenotype of a given bacterial, fungal or yeast cell and may be referred to as a "reference strain" or "reference bacterial, fungal or yeast cell". A "parental bacterial, fungal or yeast strain" may have been genetically manipulated or be a "wild-type" bacterial cell depending on the context in which the term is used. Where FucO expression is increased in non-genetically modified bacterial, fungal or yeast cells, the reference strain or reference bacterial, fungal or yeast cell will be a wild-type bacterial, fungal or yeast cell from which the bacterial, fungal or yeast cell having increased FucO activity was obtained as disclosed below.

The terms "increasing", "increase", "increased" or "increases" refers to increasing by at least 5%, for example, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99, 100% or more, a particular activity (e.g., increased FucO activity). The terms "decreasing", "decrease", "decreased" or "decreases" refers to reducing by at least 5%, for example, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99, 100% or more, a particular activity (e.g., decreased FucO activity). An increase (or decrease) in activity includes an increase (or decrease) in the rate and/or the level of a particular activity (e.g., FucO activity). "Growth" means an increase, as defined herein, in the number or mass of a bacterial, fungal or yeast cell over time.

As used herein, "FucO activity" means the reductive removal of furfural and/or 5-HMF by the action of a furfural or 5-HMF NADH-dependent oxidoreductase, such as FucO. The nucleic and amino acid sequence of the *FucO* gene (SEQ ID NO: 2) and polypeptide (SEQ ID NO: 1) are known in the art (see GenBank Accession Nos. ADT76407.1, for example and GenBank Accession No. CP002185, REGION: 3085103 - 3086251, VERSION CP002185.1 GI:315059226, Archer et al., BMC Genomics 12 (1), 9 (2011), each of which is hereby incorporated by reference in its entirety) and are provided in the sequence listing appended hereto.

In one aspect of the invention, bacterial cells having increased FucO activity can also have the activity of YqhD, YqhC and/or DkgA protein decreased or altered, as compared to the activity of YqhD, YqhC and/or DkgA protein in a reference bacterial cell or a reference bacterial cell having increased FucO activity. Activity is decreased or altered by methods known in the art, including but not limited to modification of the *yqhD, yqhC* and/or *dkgA* gene(s) (e.g. by inserting, substituting or removing nucleic acids or amino acids in the sequences encoding the genes). Thus, this aspect of the invention can also provide a bacterial cell wherein expression of FucO is increased, as compared to a reference bacterial cell and expression of the *yqhD, yqhC* and/or *dkgA* gene is/are decreased as compared to the expression of the *yqhD, yqhC* and/or *dkgA* gene in a reference bacterial cell. Expression can be decreased or altered by methods known in the art, including but not limited to modification of the *yqhD, yqhC* and/or *dkgA* gene(s) (e.g. by inserting, substituting or removing nucleic acids or amino acids in the sequences encoding the genes), inactivation or knockout of these genes. Methods for altering the activity of YqhD, YqhC and/or DkgA and inactivating the genes encoding these proteins are known in the art, see for example PCT/US2010/020051 (PCT publication WO 2010101665 A1) which is hereby incorporated by reference in its entirety.

The invention provides for a bacterial, fungal or yeast cell that has an increased resistance to furfural and further exhibit at least one of: 1) increased growth in the presence or absence of furfural as compared to a reference bacterial, fungal or yeast cell; 2) increased growth and increased production of a desired product as compared to a reference bacterial, fungal or yeast cell; 3) increased growth and increased production of a desired product, in the presence of furfural, as compared to a reference bacterial, fungal or yeast cell; 4) increased growth in the presence of a hydrolysate as compared to a reference bacterial, fungal or yeast cell; and 5) increased production of a desired product as compared to a reference bacterial, fungal or yeast cell.

The invention also provides for a bacterial, fungal or yeast cell that has an increased resistance to 5-HMF and further exhibit at least one of: 1) increased growth in the presence or absence of 5-HMF as compared to a reference bacterial, fungal or yeast cell; 2) increased growth and increased production of a desired product as compared to a reference bacterial, fungal or yeast cell; 3) increased growth and increased production of a desired product, in the presence of 5-HMF, as compared to a reference bacterial, fungal or yeast cell; 4) increased growth in the presence of a hydrolysate as compared to a reference bacterial, fungal or yeast cell; and 5) increased production of a desired product as compared to a reference bacterial, fungal or yeast cell. Methods of increasing the resistance of a bacterial, fungal or yeast cell to furfural and 5-HMF are also provided herein.

Various aspects of the invention provide for the use of a variety of hydrolysates for the production of a desired product, including but not limited to, hydrolysate derived from a biomass, a hemicellulosic biomass, a lignocellulosic biomass or a cellulosic biomass. Yet other aspects of the invention provide a bacterial, fungal or yeast cell with increased resistance to furfural, wherein the bacterial, fungal or yeast cell is capable of producing a desired product as a primary fermentation product, wherein optionally, the primary fermentation product is produced under anaerobic or microaerobic conditions.

As noted above, various aspects of the invention provide for an isolated or recombinant bacterial, fungal or yeast cell, wherein expression of FucO is increased as compared to a reference bacterial, fungal or yeast cell, and wherein the bacterial, fungal or yeast cell has increased furfural tolerance as compared to the reference bacterial, fungal or yeast cell. The invention also provides for an isolated or recombinant bacterial, fungal or yeast cell wherein the expression of FucO or the activity of FucO polypeptides is increased as compared to a reference bacterial, fungal or yeast cell, furfural tolerance is increased in the isolated or recombinant bacterial, fungal or yeast cell, as compared to the reference bacterial, fungal or yeast cell, and wherein the bacterial, fungal or yeast cell is capable of producing a desired product. In this aspect of the invention, the bacterial, fungal or yeast cell can be prepared recombinantly such that FucO activity is increased or by a process comprising the steps of: (a) growing a candidate strain of the bacterial, fungal or yeast cell in the presence of furfural; and (b) selecting bacterial, fungal or yeast cell that produces a desired product in the presence of furfural and has higher FucO activity as compared to a reference bacterial, fungal or yeast cell or parental bacterial, fungal or yeast cell.

The invention also provides for a method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with any of the isolated or recombinant bacterial, fungal or yeast cell of the invention thereby producing the desired product from a biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or an oligosaccharide source.

Further, the invention provides for a method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source in the presence of furfural comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with the isolated or recombinant bacterial, fungal or yeast cell of the invention, thereby producing the desired product from a biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or an oligosaccharide source.

The subject application also provides the following non-limiting embodiments:

1. An isolated bacterial, fungal or yeast cell having increased NADH-dependent propanediol oxidoreductase (FucO) activity as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF).

2. The isolated bacterial, fungal or yeast cell of embodiment 1, wherein said bacterial, fungal or yeast cell produces a desired product or has been genetically engineered to produce a desired product selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

3. The isolated bacterial, fungal or yeast cell of embodiments 1-2, wherein said bacterial, fungal or yeast cell exhibits increased production of said desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

4. The isolated bacterial cell of embodiments 1-3, wherein:

a) expression of the yqhD gene is reduced in said bacterial cell as compared to a reference bacterial cell;

b) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

c) expression of the yqhD gene, yqhC gene and/or the dkgA gene are reduced in said bacterial cell as compared to expression in a reference bacterial cell;

d) expression of the yqhC gene is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

e) expression of the yqhD gene, the yqhC gene and the dkgA genes is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

f) the yqhD gene is not expressed or is deleted in said bacterial cell;

g) the yqhD gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

h) the yqhC gene or yqhD gene, the yqhC gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

i) the yqhC gene is not expressed or is deleted in said bacterial cell;

j) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

k) the dkgA gene is not expressed in said bacterial cell;

l) the activity of the yqhD gene, yqhC gene and/or the dkgA gene product(s) are reduced in said bacterial cell as compared to expression in a reference bacterial cell; or

m) the activity of the yqhC gene product is reduced in said bacterial cell as compared to expression in a reference bacterial cell.

5. The isolated bacterial cell of embodiment 4, wherein the activity of YqhD protein is reduced in said bacterial cell as compared to a reference bacterial cell.

6. The isolated bacterial cell of embodiment 4, wherein the activity of the YqhD protein and the activity of the DkgA protein is reduced in said bacterial cell as compared to a reference bacterial cell.

7. The isolated bacterial cell of embodiment 4, wherein the activity of the YqhC protein is reduced in said bacterial cell as compared to a reference bacterial cell.

8. The isolated bacterial cell of embodiment 4, wherein regulation of the expression of the yqhD gene is altered to reduce yqhD expression as compared to a reference bacterial cell.

9. The isolated bacterial cell of embodiment 4, wherein regulation of the expression of the yqhD gene and regulation of expression of the dkgA gene is altered to reduce yqhD and dkgA expression in said bacterial cell as compared to expression in a reference bacterial cell.

10. The isolated bacterial cell of embodiment 4, wherein regulation of expression of the *yqhC* gene is altered to reduce yqhC expression in said bacterial cell as compared to expression in a reference bacterial cell.

11. The isolated bacterial cell of embodiment 4, wherein the *yqhC gene, yqhD gene, dkgA* gene or any combination thereof is/are deleted in said bacterial cell.

12. The isolated bacterial cell of embodiment 4, wherein there is a change in the activity of the yqhD gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

13. The isolated bacterial cell of embodiment 4, wherein there is a change in the activity of the dkgA gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

14. The isolated bacterial cell of embodiment 4, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an antisense RNA as compared to a reference bacterial cell.

15. The isolated bacterial cell of embodiment 4, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an siRNA as compared to a reference bacterial cell.

16. The isolated bacterial, fungal or yeast cell of any preceding embodiment, wherein FucO activity is increased by:

a) expressing the *FucO* gene in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of the *FucO* gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of the *FucO* gene native promoter with a promoter that increases the level of gene expression in a bacterial cell; or

d) the FucO enzyme is mutated to increase catalytic efficiency or reduce its Km.

17. An isolated bacterial, fungal or yeast cell having increased FucO activity wherein said bacterial, fungal or yeast cell is capable of producing a desired product, or which has been genetically engineered to produce a desired product, and wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the bacterial, fungal or yeast cell in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, furfural and/or or 5-hydroxymethylfurfural (5-HMF), preferably about 15 mM furfural or about 15 mM to about 30 mM 5-HMF.

18. The isolated bacterial, fungal or yeast cell of embodiment 17, wherein said cells are grown in the presence of furfural (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM furfural).

19. The isolated bacterial, fungal or yeast cell of embodiment 17, wherein said cells are grown in the presence of 5-HMF (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM 5-HMF).

20. The isolated bacterial, fungal or yeast cell of embodiments 17-19, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

21. A method of growing a bacterial, fungal or yeast cell comprising culturing a bacterial, fungal or yeast cell according to any one of embodiments 1-20 under conditions that allow for the growth of said bacterial, fungal or yeast cell.

22. A method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with the isolated bacterial, fungal or yeast cell according to any one of embodiments 1-20 and producing said desired product by fermenting said biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source in the presence of said bacterial, fungal or yeast cell.

23. The method of embodiment 21 or 22, wherein the bacterial, fungal or yeast cell produces a desired product, or has been genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

24. The method according to embodiment 21, 22 or 23, wherein said bacterial, fungal or yeast cell exhibits increased production of a desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

25. A method of increasing furfural and/or 5-hydroxymethylfurfural (5-HMF) resistance in a bacterial, fungal or yeast cell comprising increasing NADH-dependent propanediol oxidoreductase (FucO) activity in said bacterial, fungal or yeast cell, as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast

cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF).

26. The method according to embodiment 25, wherein:

a) expression of the yqhD gene is reduced in said bacterial cell as compared to a reference bacterial cell;

b) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

c) expression of the yqhD gene, yqhC gene and/or the dkgA gene are reduced in said bacterial cell as compared to expression in a reference bacterial cell;

d) expression of the yqhC gene is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

e) expression of the yqhD gene, the yqhC gene and the dkgA genes is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

f) the yqhD gene is not expressed or is deleted in said bacterial cell;

g) the yqhD gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

h) the yqhC gene or yqhD gene, the yqhC gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

i) the yqhC gene is not expressed or is deleted in said bacterial cell;

j) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

k) the dkgA gene is not expressed in said bacterial cell;

l) the activity of the yqhD gene, yqhC gene and/or the dkgA gene product(s) are reduced in said bacterial cell as compared to expression in a reference bacterial cell; or

m) the activity of the yqhC gene product is reduced in said bacterial cell as compared to expression in a reference bacterial cell.

27. The method according to embodiment 26, wherein the activity of YqhD protein is reduced in said bacterial cell as compared to a reference bacterial cell.

28. The method according to embodiment 26, wherein the activity of the YqhD protein and the activity of the DkgA protein in said bacterial cell is reduced in said bacterial cell as compared to a reference bacterial cell.

29. The method according to embodiment 26, wherein the activity of the YqhC protein is reduced in said bacterial cell as compared to a reference bacterial cell.

30. The method according to embodiment 26, wherein regulation of the expression of the yqhD gene is altered to reduce yqhD expression in said bacterial cell as compared to a reference bacterial cell.

31. The method according to embodiment 26, wherein regulation of the expression of the yqhD gene and regulation of expression of the dkgA gene is altered to reduce yqhD and dkgA expression in said bacterial cell as compared to expression in a reference bacterial cell.

32. The method according to embodiment 26, wherein regulation of expression of the *yqhC* gene is altered to reduce yqhC expression in said bacterial cell as compared to expression in a reference bacterial cell.

33. The method according to embodiment 26, wherein the *yqhC gene, yqhD gene, dkgA* gene or any combination thereof is/are deleted.

34. The method according to embodiment 26, wherein there is a change in the activity of the yqhD gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

35. The method according to embodiment 26, wherein there is a change in the activity of the dkgA gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

36. The method according to embodiment 26, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an antisense RNA as compared to a reference bacterial cell.

37. The method according to embodiment 26, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an siRNA as compared to a reference bacterial cell.

38. The method according to any one of embodiments 25-37, wherein FucO activity is increased by:

a) expressing the *FucO* gene in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of the *FucO* gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of the *FucO* gene native promoter with a promoter that increases the level of gene expression in a bacterial, fungal or yeast cell; or

d) the FucO enzyme is mutated to increase catalytic efficiency or reduce its Km.

39. The method according to embodiment 25, wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the bacterial, fungal or yeast cell in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

40. The method according to embodiment 39, wherein said cell is grown in the presence of furfural.

41. The method according to embodiment 39, wherein said cell is grown in the presence of 5-HMF.

42. The method according to any one of embodiments 39-41, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

43. The isolated bacterial cell or method according to any one of embodiments 1-42, wherein said bacterial cell is a Gram-negative or a Gram-positive bacterial cell.

44. The isolated bacterial cell or method according to embodiment 43, wherein the Gram-negative bacterial cell is a bacterial cell selected from the genera of *Escherichia, Zymomonas, Acinetobacter, Gluconobacter, Geobacter, Shewanella, Salmonella, Enterobacter* or *Klebsiella* and the Gram-positive bacteria is a bacterial cell selected from the genera of *Bacillus, Clostridium, Corynebacterial cell, Lactobacillus, Lactococcus, Oenococcus, Streptococcus* and *Eubacterial cell.*

45. The isolated bacterial cell or method according to embodiment 44, wherein the bacterial cell is *Escherichia coli* or *Klebsiella oxytoca*.

46. The isolated bacterial cell or method according to embodiment 43, wherein said bacterial cell is selected from *Thermoanaerobes, Bacillus spp.*, *Paenibacillus* spp. or *Geobacillus* spp.

47. The isolated yeast cell or method according to any one of embodiments 1-42, wherein said yeast cell is a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, or *Yarrowia* cell.

48. The isolated yeast cell or method according to embodiment 47, wherein said yeast cell is *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis,* or *Yarrowia lipolytica.*

49. The isolated fungal cell or method according to embodiments 1-42, wherein said fungal cell is a *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes*, or *Trichoderma* cell.

50. The isolated fungal cell or method according to embodiment 49, wherein said fungal cell is an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei,* or *Trichoderma viride* cell.

51. The isolated bacterial, fungal or yeast cell according to any one of embodiments 1-20 or 43-50, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural and/or 5-HMF.

52. An isolated bacterial, fungal or yeast cell having increased NADH-dependent furfural or 5-HMF oxidoreductase activity as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased NADH-dependent furfural or 5-HMF oxidoreductase activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF).

53. The isolated bacterial, fungal or yeast cell of embodiment 52, wherein said bacterial cell produces a desired product, or has been genetically engineered to produce a

desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

54. The isolated bacterial, fungal or yeast cell of embodiments 52-53, wherein said bacterial, fungal or yeast cell exhibits increased production of said desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

55. The isolated bacterial, fungal or yeast cell of embodiments 52, 53 or 54, wherein FucO activity is increased by:

a) expressing one or more NADH-dependent furfural or 5-HMF oxidoreductase in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of a NADH-dependent furfural or 5-HMF oxidoreductase gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of a native promoter for a NADH-dependent furfural or 5-HMF oxidoreductase with a promoter that increases the level of gene expression in a bacterial, fungal or yeast cell; or

d) a NADH-dependent furfural or 5-HMF oxidoreductase enzyme is mutated to increase catalytic efficiency or reduce its Km.

56. An isolated bacterial, fungal or yeast cell having increased FucO activity wherein said bacterial, fungal or yeast cell is capable of producing a desired product, or has been genetically engineered to produce a desired product, and wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the bacterial, fungal or yeast cell in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product, or has been genetically engineered to produce a desired product and produce said product, in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, furfural and/or or 5-hydroxymethylfurfural (5-HMF), preferably about 15 mM furfural or about 15 mM to about 30 mM 5-HMF.

57. The isolated bacterial, fungal or yeast cell of embodiment 56, wherein said cells are grown in the presence of furfural (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM furfural).

58. The isolated bacterial, fungal or yeast cell of embodiment 56, wherein said cells are grown in the presence of 5-HMF (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM 5-HMF).

59. The isolated bacterial, fungal or yeast cell of embodiments 56-58, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

60. A method of growing a bacterial, fungal or yeast cell comprising culturing a bacterial, fungal or yeast cell according to any one of embodiments 52-59 under conditions that allow for the growth of said bacterial, fungal or yeast cell.

61. A method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with the isolated bacterial, fungal or yeast cell according to any one of embodiments 52-59 and producing said desired product by fermenting said biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source in the presence of said bacterial, fungal or yeast cell.

62. The method of embodiment 60 or 61, wherein the bacterial, fungal or yeast cell produces a desired product, or has been genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

63. The method according to embodiment 60, 61 or 62, wherein said bacterial, fungal or yeast cell exhibits increased production of a desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

64. A method of increasing furfural and/or 5-hydroxymethylfurfural (5-HMF) resistance in a bacterial, fungal or yeast cell comprising increasing NADH-dependent furfural or 5-HMF oxidoreductase activity in said bacterial, fungal or yeast cell, as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF) and produces a desired product, as disclosed herein, or has been genetically engineered to produce a desired product.

65. The isolated bacterial cell or method according to any one of embodiments 52-64, wherein said bacterial cell is a Gram-negative or a Gram-positive bacterial cell.

66. The isolated bacterial cell or method according to embodiment 65, wherein the Gram-negative bacterial cell is a bacterial cell selected from the genera of *Escherichia, Zymomonas, Acinetobacter, Gluconobacter, Geobacter, Shewanella, Salmonella, Enterobacter* or *Klebsiella* and the Gram-positive bacteria is a bacterial cell selected from the genera of *Bacillus, Clostridium, Corynebacterial cell, Lactobacillus, Lactococcus, Oenococcus, Streptococcus* and *Eubacterial cell.*

67. The isolated bacterial cell or method according to embodiment 66, wherein the bacterial cell is *Escherichia coli* or *Klebsiella oxytoca.*

68. The isolated bacterial cell or method according to embodiment 66, wherein said bacterial cell is selected from *Thermoanaerobes, Bacillus spp.*, *Paenibacillus* spp. or *Geobacillus* spp.

69. The isolated yeast cell or method according to any one of embodiments 52-64, wherein said yeast cell is a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, or *Yarrowia* cell.

70. The isolated yeast cell or method according to embodiment 69, wherein said yeast cell is *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis,* or *Yarrowia lipolytica.*

71. The isolated fungal cell or method according to embodiments 52-64, wherein said fungal cell is a *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes*, or *Trichoderma* cell.

72. The isolated fungal cell or method according to embodiment 71, wherein said fungal cell is an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides,*

Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or *Trichoderma viride* cell.

73. The isolated bacterial, fungal or yeast cell or method according to any one of embodiments 52-72, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural and/or 5-HMF.

74. The method of embodiments 60, 61, 62 or 63, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural.

75. The method of embodiments 60, 61, 62 or 63, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM 5-HMF.

76. The isolated bacterial, fungal or yeast cell or method according to any one of the preceding embodiments, wherein said bacterial, fungal or yeast cell has been genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

The terms comprise, comprises, comprising, having and containing are open-ended terms and can be used interchangeably with consisting, consists or consisting essentially of throughout the subject application and claims.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1—ALTERATION OF FucO ACTIVITY FOR THE PRODUCTION OF A DESIRED PRODUCT

Strains, media and growth conditions

Strains, plasmids and primers used in this study are listed in Table 1. During strain constructions, cultures were grown aerobically in Luria broth containing 20 g liter^{-1} glucose or 50 g liter^{-1} arabinose. Ampicillin (50 mg liter^{-1}), kanamycin (50 mg liter^{-1}), or chloramphenicol (40 mg liter^{-1}) were added as appropriate. Red recombinase technology (Gene Bridges GmbH, Dresden, Germany) was used to facilitate chromosomal integration as previously described (17, 19, 40, 41). All constructions were verified by DNA sequencing.

Strain LY180 was previously engineered for ethanol production (29). A derivative of LY180 was constructed with a deletion in the *yqhD* gene (29) encoding a NADPH-dependent furfural reductase activity, now designated strain EM322. Strain EMFR9 is a mutant of LY180 selected for furfural resistance by serial cultivation in AM1-xylose medium containing added furfural (29). This strain was found to contain an IS10 insertion in an adjacent regulatory gene (*yqhC*) that silenced the expression of *yqhD* (37).

Strain MM160 is a derivative of strain LY180 selected for resistance to hemicellulose hydrolysates of sugar cane that contains furfural and other inhibitors (13). Partial sequencing of this strain revealed a nonsense mutation, truncating the YqhD protein after the methionine at position 245. Additional genetic modifications were made in strain MM160 to engineer new strains for L- and D-lactate production. The *E. coli ldhA* gene was restored in the chromosome (D-lactate dehydrogenase) as previously described (17, 19). The *Zymomonas mobilis* ethanol pathway (*pdc*, *adhA*, and *adhB*) and the *Pseudomonas putida* esterase gene *estZ* with an adjacent FRT site were then deleted to produce XW043 for D-lactate production. The native *ldhA* ORF in XW043 was replaced with *Pediococcus acetolactici ldhL* ORF (L-lactate dehydrogenase) from *E. coli* TG108 (15) to produce strain XW042 for the production of L-lactate.

Lactate strains were grown in small fermentation vessels (500-ml; 300 ml broth) at 37°C (150 rpm) in AM1 mineral salts medium (24) containing 50 g liter^{-1} xylose or 1 mM betaine and 100 g liter^{-1} xylose. Fermentations were maintained at pH 7.0 (lactate production) by the automatic addition of KOH. Lactate strains were serially transferred for approximately 500 generations to improve xylose utilization and lactate productivity. The resulting strains were designated XW068 (D-lactate) and XW059 (L-lactate).

Furfural toxicity and furfural reduction in vivo

Furfural toxicity was measured in tube cultures (13 mm by 100 mm) containing 4 ml of AM1 medium with 50 g liter^{-1} xylose, 12.5 mg liter^{-1} ampicillin, furfural, and other supplements as indicated (28, 29). Cultures were inoculated to an initial density of 22 mg dcw liter^{-1}. IPTG (0.1 mM) was included for *fucO* induction. Cell mass was measured at 550 nm after incubation for 48 h (37°C).

In vivo furfural reduction was measured during incubation in AM1 medium containing 10 mM furfural and 50 g liter^{-1} xylose. Cells were pre-incubated with chloramphenicol (40 mg liter^{-1}) for 1 h to arrest growth (0.88 mg dcw cells ml^{-1}), prior to the addition of furfural. Furfural concentration was measured as previously described using a Beckman spectrophotometer DU800 (27).

Plasmids for *fucO* expression

Plasmids were constructed for the controlled expression of *fucO*. The DNA sequence of *fucO* (coding region, ribosome binding site, and terminator) were amplified from *E. coli* LY180 by PCR and cloned between the EcoRI and BamHI sites of pTrc99A to produce pLOI4319. This plasmid was used for the inducible expression of *fucO*. The FucO coding region was cloned into pET15b to produce pLOI4322. This enzyme was purified as a his-tagged product.

FucO assay and purification

Cultures were grown overnight to a cell density of approximately 0.66 mg dcw ml^{-1} (37°C) in closed tubes containing 20 ml AM1 (50 g liter^{-1} xylose, 0.1 mM IPTG and 12.5 mg liter^{-1} ampicillin). Cells were harvested by centrifugation (7,000 g for 5 min, 4°C), washed twice with 10 ml of cold sodium phosphate buffer (50 mM pH 7.0), resuspended to a cell density of 4.4 mg dcw ml^{-1}, and disrupted in buffer containing 1 mM dithiothreitol using a Fastprep-24 (MP Biomedicals, Solon, OH). After clarification at 13,000 g (10 min, 4°C), protein concentration was determined using a BCA^{TM} Protein Assay Kit (Thermo Scientific, Rockford, IL). Furfural-dependent reduction was measured using NADH and NADPH by monitoring the decrease in absorbance at 340 nm (extinction coefficient of NADH of 6,220 M^{-1} cm^{-1}; extinction coefficient of NADPH of 6,020 M^{-1} cm^{-1}). Reaction mixtures contained 200 mM phosphate buffer (pH 7.0), 10 mM furfural, and 0.2 mM NADH or NADPH. NADH-dependent and NADPH-dependent reduction of 5-HMF (10 mM) was measured in a similar fashion.

For the purification of his-tagged FucO, BL21(pLOI4322) was grown in Luria broth at 37°C. When the culture density reached 0.35 g dcw $liter^{-1}$, IPTG (0.1 mM) was added to induce overexpression. After incubation for 4 h, cells were harvested (7,000 g for 5 min, 4°C), washed once with 10 mM Tris-HCl (pH 7.1), and lysed using a French pressure cell. After clarification at 30,000 g (1 h, 4°C), crude extracts were passed through a 0.22 µm polyvinylidene fluoride (PVDF) filter, and further purified using a 1-ml HiTrap^{TM} nickel column (GE Healthcare, Piscataway, NJ). Purified enzyme was dialyzed in 100 mM phosphate buffer using a Thermo Slide-A-Lyzer and quantified using the BCA^{TM} Protein Assay Kit (Thermo Scientific, Rockford, IL). A single band was observed in a sodium dodecyl sulfate-polyacrylamide gel.

Effect of *fucO* expression on fermentation

Seed pre-cultures of strains containing pTrc99A or pLOI4319 were grown from plates using sealed culture tubes containing AM1 medium (20 g $liter^{-1}$ xylose, 12.5 mg $liter^{-1}$ ampicillin). MOPS buffer (100 mM; pH 7.0) was included for seed cultures of lactate strains XW068 and XW059. After incubation for 16 h, pre-inocula were diluted into 500-ml fermentation vessels containing 300 ml AM1 media (100 g $liter^{-1}$ xylose, 1 mM betaine, 0.1 mM IPTG, 12.5 µg ml^{-1} ampicillin) to provide a starting density of 13.2 mg dcw. After 24 h growth, these seed cultures were used to provide a starting inoculum for batch fermentations (AM1 medium, 100 g $liter^{-1}$ xylose, 12.5 µg ml^{-1} ampicillin, 0.1 mM IPTG, 13.2 mg dcw initial density, and furfural). Fermentations were maintained at pH 6.5 (ethanol) or pH 7.0 (lactate) by the automatic addition of KOH as previously described (15, 29). Ethanol was measured using an Agilent 6890N gas chromatograph (Palo Alto, CA) equipped with flame ionization detectors and a 15-meter HP-Plot Q Megabore column. Furfural concentration was monitored using a Beckman DU spectrophotometer (27, 29). Organic acids and xylose were measured by high-performance liquid chromatography (15).

Results:

FucO has NADH-dependent furan reductase activity.

The *fucO* gene was cloned (pLOI4319) and transformed into LY180. Cell lysates were compared to LY180 with vector alone (Figs. 2A and 2B). Control lysates with vector exhibited low levels of NADPH-dependent furan reductase activity for both furfural and 5-HMF, and even lower levels of NADH-depended activity (Fig. 2B). Expression of *fucO* from pLOI4319 resulted in a 60-fold increase in NADH-dependent furfural reductase activity and a 6-fold increase in NADH-dependent 5-HMF reductase activity, but no increase in NADPH-dependent activity.

His-tagged *fucO* was overexpressed in BL21(λDE3) and purified to homogeneity. This protein catalyzed the NADH-specific reduction of furfural and 5-HMF with apparent K_m values of 0.4 ± 0.2 mM and 0.7± 0.3 mM, respectively. Apparent V_{max} values for furfural and 5-HMF were 1.9 ± 0.4 and 0.30 ± 0.05 μmol min^{-1} mg $protein^{-1}$, respectively. No NADPH-dependent furfural or 5-HMF reductase activity was observed with the purified enzyme. The apparent K_m value for furfural (0.4 mM) with FucO was significantly lower than that of YqhD (9 mM furfural) (29).

Expression of *fucO* increased furfural metabolism in vivo and increased furfural tolerance in tube cultures.

IPTG-induced expression of *fucO* in LY180(pLOI4319) increased the in vivo specific activity (whole cell) for furfural reduction by 4-fold as compared to the control strain, LY180(pTrc99A) containing empty vector (Fig. 2C). This increase in furfural reduction activity was accompanied by a 50% increase in the minimum inhibitory concentration, from 10 mM furfural to 15 mM furfural (Fig. 2D). Although a smaller increase in activity was observed without inducer, this change was not sufficient to affect the minimum inhibitory concentration.

Combined effects of *fucO* overexpression and media supplements.

Based on the proposed model for furfural inhibition of growth (Fig. 1), overexpression of *fucO* would be expected to have a combined benefit with other approaches that increase the availability of NADPH. Previous studies (28, 29) have shown that furfural tolerance can be increased by the addition of complex nutrients, cysteine (decreased biosynthetic demand for NADPH), or by the replacement of xylose with glucose (increased

NADPH production). LY180(pTrc99A) and LY180(pLOI4319) were unable to grow in the presence of 15 mM furfural (Fig. 3A and 3B) without supplements. With supplements, growth was limited and was further increased by the expression of *fucO* (pLOI4319). With 10 mM furfural, replacement of xylose with glucose substantially restored growth of the control strain LY180(pTrc99A). Expression of *fucO* from pLOI4319 provided a small additional benefit with glucose (Fig. 3C).

Expression of *fucO* increased ethanol production in the presence of furfural.

The effect of *fucO* expression on furfural tolerance was examined during batch fermentations of xylose to ethanol (Fig. 4). Strain LY180(pLOI4319) was unable to grow in the presence of 15 mM furfural, but metabolized furfural at a faster rate than the control containing vector alone. A derivative of LY180 was previously constructed in which *yqhD* was deleted, denoted strain EM322 (29). After a 24-h lag during which most of the furfural was metabolized, EM322 began to grow and ferment xylose to ethanol. Expression of *fucO* in EM322(pLOI4319) increased the rate of furfural metabolism, decreased the growth lag, and increased the rate of xylose fermentation to ethanol. Although ethanol production with furfural was improved by expression of *fucO*, EM322(pLOI4319) still required longer fermentation times than control strains without furfural. Final ethanol yields (100 g liter^{-1} xylose) for EM322(pLOI4319), and EM322(pTrc99A) with furfural (15 mM) were similar to those for strains without furfural, approximately 90% of the theoretical maximum. A mutation in *yqhD* and expression of *fucO* were both required for the optimal fermentation of broth containing15 mM furfural.

Expression of *fucO* increased lactate production in the presence of furfural

E. coli strain MM160 is a furfural-resistant derivative of strain LY180. This strain was selected for resistance to hemicellulose hydrolysates of bagasse that contain furfural and other inhibitors (13). Sequencing of a PCR fragment of *yqhD* from this strain revealed a nonsense mutation (G to A in codon 246, forming a TAG stop codon) that truncates 30% of the YqhD protein. Strain MM160 was re-engineered for D-lactate production and designated strain XW068. This strain was used to examine the effects of *fucO* expression on lactate production in xylose broth containing furfural. With XW068 (pTrc99A), growth and lactate production from xylose were slowed by the addition of 10 mM furfural (Figs. 5A, 5B, 5C). Expression of *fucO* in XW068(pLOI4319) substantially improved both in comparison to the vector control. With 15 mM furfural, only XW068(pLOI4319) was able to grow and ferment xylose (Figs. 5D, 5E, 5F). Although the control with vector alone continued to metabolize furfural during incubation, minimal growth and lactate production occurred after 120 h. The final yield of D-lactate for XW068 (pLOI4319) with 15 mM furfural was near that of the control grown without furfural, approximately 85% of the theoretical maximum. Similar beneficial effects of *fucO* overexpression were also observed with XW059 which was engineered from MM160 for L-lactate production (data not shown). Improved tolerance to the presence of furfural was observed by the overexpression of *fucO* alone in cells and the cells produced lactate. Introduction of the mutation into *yqhD* further increased resistance to furfural and also increased production of lactate.

Discussion:

Furfural, the dehydration product of pentose sugars, is an important microbial inhibitor that is formed during dilute acid hydrolysis of hemicelluloses (1, 31). Diverse approaches have been explored for furfural removal such as lime addition (pH 10) (1, 25, 26) and the selection of resistant mutants (1, 21, 29). Developing biocatalysts that are more furfural tolerant would be helpful for the production of renewable products from inedible feedstocks.

Furfuryl alcohol is known to be less toxic than furfural (38, 39). Thus an effective microbial furfural reduction system has the potential to increase furfural resistance. Furfural-resistant strains of *S. cerevisiae* have been isolated (2, 20, 22, 23) and found to exhibit increased expression of aldehyde reductases that may contribute to tolerance. In *E. coli*, many oxidoreductases were also induced by furfural but none originally tested were found to reduce toxicity when overexpressed in the parent strain (28, 29). Two independent, furfural-resistant mutants of *E. coli* were investigated and both were found to have mutations affecting the furfural-inducible *yqhD* gene encoding a furfural reductase activity. In EMFR9, *yqhD* expression was silenced by an IS10 insertion into the adjacent regulatory gene (*yqhC*) (37). In MM160, *yqhD* was truncated by a nonsense mutation ((13); this study). Deletion of *yqhD* in the parent strain increased furfural tolerance and overexpression of *yqhD* in the mutants restored furfural sensitivity (29). A mutation in *yqhD* alone (EM322) is sufficient to permit growth in xylose broth containing 10 mM furfural (29). The negative effect of YqhD has been attributed to an unusually low Km for NADPH (8 μM), starving essential biosynthetic reactions by depletion of the NADPH pool (Fig. 1).

The discovery of furfural reductase activity in FucO offered an alternative route for furfural reduction to the less toxic alcohol using NADH, an abundant reductant during fermentation. Furfural reduction by this enzyme removed substrate from YqhD and other NADPH-furfural reductases in *E. coli* strains and increased furfural tolerance. Additionally, the combination of *fucO* expression and silencing of *yqhD* permitted fermentation in xylose broth containing 15 mM furfural, a concentration similar to that present in hemicellulose hydrolysates of woody biomass (13, 14).

FucO belongs to the iron-activated group III dehydrogenase family (33). This enzyme catalyzes the interconversion between L-lactaldehyde and L-1,2-propanediol during the anaerobic dissimilation of fucose (6, 10) and aerobic growth on L-1,2-propanediol (9). FucO has been shown to utilize a broad spectrum of substrates including glycerol, ethylene glycol, L-lactaldehyde, glycoaldehyde, acetaldehyde, glyceraldehyde, propionaldehyde, and methylglyoxal (5, 6, 11) but was not previously known to reduce furans. The sequence of this gene is similar to the iron-containing alcohol dehydrogenase II from *Zymomonas mobilis* and ADH4 from *S. cerevisiae* (11). Although the amino acid identities are quite low, the crystal structure of FucO is very similar to that of YqhD (32, 36), both of which metabolize furfural. FucO and YqhD are each composed of two subunits, with an α/β Rossman nucleotide binding N-terminal domain and an all-α helical C-terminal domain. FucO activity has a K_m value for furfural of 0.4 mM, much lower than that of YqhD (9 mM) (29). The V_{max} of FucO for furfural is only 10% that for L-lactaldehyde (20 μmol min^{-1} mg $protein^{-1}$, 0.035 mM) indicating a strong preference for the native substrate (6). High levels of FucO appear to be needed to increase furfural tolerance in *E. coli*, consistent with the low catalytic rate of furfural reduction. The dehydration product of hexose sugars, 5-HMF, was also metabolized by the FucO enzyme (Fig. 2).

Overexpression of an NADH-dependent furfural reductase provides a detoxification strategy that may be generally useful for other enteric microbial catalysts (Fig. 1). NADPH-dependent reductases are widely used for detoxification processes and appear best suited for aerobic growth where NADPH is more abundant. NADPH-dependent activities could be replaced with NADH-dependent activities in biocatalysts designed for anaerobic fermentation products. Our studies have demonstrated the utility of this approach both for ethanol production and lactate production using engineered strains of *E. coli* (Figs. 4 and 5). When combined with other approaches that increase the availability of NADPH, overexpression of *fucO* can provide a further benefit for furfural tolerance. An analogous strategy that minimizes the depletion of NADPH pools during detoxification process may be generally useful for other toxic agents in lignocellulosic sugar streams and with other organisms.

EXAMPLE 2—ALTERATION OF FucO ACTIVITY FOR THE PRODUCTION OF SUCCINATE

In this example, succinate-producing biocatalysts that can ferment xylose effectively and have increased resistance to furfural, a toxic compound present in xylose-rich hemicellulose hydrolysates (dilute acid hydrolysis, 160-190C) were constructed. Strain KJ122 is an excellent biocatalysts designed to ferment glucose to succinate (17, 40, 42). In this study, we adapted strains that grow well on xylose. This strain has been further engineered for furfural tolerance by deleting or silencing the *yqhD* gene encoding a NADPH-dependent furfural reductase, an activity that depletes the NADPH pool needed for biosynthesis and growth. In addition, we demonstrate a second gene *fucO* that alone or in combination with the *yqhD* deletion provide increased tolerance to furfural.

The fucO gene normally functions in fucose degradation. We have discovered that the FucO enzyme can use furfural and 5-hydroxymethylfurfural as a substrate. Both are reduced to their respective alcohols using NADH as the reductant. NADH is an abundant cofactor during fermentative growth.

Materials and Methods

Strains, plasmids media and growth conditions

Strains, plasmids and primers used in this example are listed in Table 2. Cultures were grown aerobically in Luria broth containing 2% (w/v) glucose or 5% (w/v) arabinose during strain constructions. Ampicillin (50 mg liter^{-1}), kanamycin (50 mg liter^{-1}), or chloramphenicol (40 mg liter^{-1}) were added as appropriate. KJ122 was previously engineered for efficient succinate production from glucose (17, 43). After approximately 50 generations (approximately 10 sequential transfers) in AM1 100 g liter^{-1} xylose media, this strain fermented xylose to succinate. A clone was isolated at the end of this adaptation and designated strain XW055. Strain XW055 ferments xylose to succinate as efficiently as KJ122 ferments glucose to succinate.

Two genes

The *yqhD* ORF in XW055 was deleted to generate strain XW056. The E. coli *fucO* ORFs were integrated under *adhE* promoters and ribosomal binding sites into the chromosomes of XW055 and XW056 to construct strains XW057 and XW081. Red recombinase technology (Gene Bridges GmbH, Dresden, Germany) was used to facilitate chromosomal integration as previously described (17, 19, 40, 41). The plasmids used in strain construction were generated as previously described (17, 19, 40, 41). CloneEZ® PCR Cloning Kit (GenScript, Piscataway, NJ, USA) was used to construct the plasmid pLOI5209 containing *fucO* ORF exactly replacing *adhE* ORF.

Furfural toxicity measurement

Furfural toxicity was measured using tube cultures (13 by 100 mm) containing 4 ml of AM1 media with 50 g liter^{-1} (w/v) xylose, 50 mM $KHCO_3$, 100 mM MOPS pH 7, and 10 mM furfural. Cultures were inoculated to an initial density of 44 mg dcw liter^{-1}. Cell mass was measured at 550 nm after incubation for 48 h at 37°C.

Results and Discussion

With *fucO* integrated under *adhE* promoter, the modified strain XW057 showed more furfural resistance than its parent strain XW055 (Fig. 6). The deletion of *yqhD* alone only showed very limited benefit in furfural resistance (Fig. 6). Interestingly, the two genetic modifications together, deletion of *yqhD* and *fucO* integration, showed the greatest resistance to furfural and there was an approximately 10-fold increase in cell mass compared to the parent strain XW055 in the presence of 10 mM furfural (Fig. 6).

This result is consistence with the discoveries obtained in *E. coli* strains engineered for ethanol production (EM322) and lactate production (XW0059 and XW068). XW055 is derived from *E. coli* C (ATCC8739) and EM322, XW059 and XW068 are derived from *E. coli* W (ATCC9637). This suggests *fucO* expression together with *yqhD* deletion as a general furfural detoxification approach that can be applied in different *E. coli* strains producing different products.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

Table 1: Bacterial strains, plasmids, and primers

Strains, plasmids, or primers	**Relevant characteristics**	**Reference of source**
Strains		
LY180	Δ*frdBC::(Zm frg celY*$_{Ec}$), Δ*ldhA::(Zm* frg *casAB*$_{Ko}$*), adhE::(Zm* frg *estZ*$_{Pp}$ FRT), Δ*ackA*::FRT, *rrlE::(pdc adhA adhB* FRT), Δ*mgsA*::FRT	(29)
EM322	LY180 Δ*yqhD*::FRT	(29)
BL21 (λDE3)	F$^-$ *ompT gal dcm lon hsdS*$_B$*(r*$_B^-$ *m*$_B^-$*)* λ(DE3 [*lacI lacUV5*-T7 gene 1 *ind1 sam7 nin5*])	Promega, Madison, WI
MM160	Hydrolysate resistant derivative of LY180, *yqhD* frame shift	(13)
XW042	MM160 *ldhA*::*ldhL*, Δ(*rrlE::[pdc adhA adhB* FRT]), Δ(*Zm frg estZ*$_{Pp}$ FRT) L-lactate production	This study
XW043	MM160 *ldhA* restored, *Δ(rrlE::[pdc adhA adhB* FRT]), Δ(*Zm frg estZPp* FRT) D-lactate production	This study
XW059	XW042 after serial transfer with xylose L-lactate production	This study
XW068	XW043 after serial transfer with xylose D-lactate production	This study
Plasmids		
pTrc99A	pTrc *bla* oriR *rrnB lacI*^{q}	(4)
pCR2.1	TOPO cloning vector	Invitrogen
pLOI4162	PacI flanked *cat-sacB* cassette	(17)
pET15b	T7 expression vector	Novagen
fucO cloning and expression		
pLOI4319	*fucO* in pTrc99A	This study
pLOI4322	*fucO* in pET15b	This study
Deletion of (*rrlE*::[*pdc adhA adhB* FRT])		
pLOI4780	*pdc-adhA-adhB from* LY180 cloned into pCR2.1-TOPO vector	This study
pLOI4781	*cat-sacB* cassette cloned into *pdc-adhA-adhB* in pLOI4780	This study
pLOI4782	PacI digestion of pLOI4781; self-ligated to delete *pdc-adhA-adhB*	This study
Deletion of (*adhE::[Zm frg estZ*$_{Pp}$FRT])		
pLOI4811	*adhE::(Zm frg estZPp* FRT*)* region cloned into pCR2.1-TOPO vector	This study
pLOI4824	*cat-sacB* cassette cloned into the *adhE::(Zm frg estZPp* FRT*)* region of pLOI4811	This study
pLOI5167	*E. coli adhE* ORF and its adjacent regions cloned into pCR2.1 TOPO vector	This study
pLOI5168	*cat-sacB* cassette cloned into *adhE* in pLOI5167	This study
pLOI5169	PacI digestion of pLOI5168; self-ligated to delete *adhE*	This study

Table 1: Bacterial strains, plasmids, and primers

ldhA restoring		
pLOI4652	*ldhA* (PCR) from *E. coli* cloned into the pCR2.1-TOPO vector	(40)
pLOI4653	*cat-sacB* cassette cloned into *ldhA* of pLOI4652	(40)
ldhL integration		
pLOI5161	*ldhA* ORF and its adjacent regions cloned into pCR2.1 TOPO vector	This study
pLOI5174	*ldhL* ORF from TG108 was cloned and used to replace the *ldhA* ORF in pLOI5161	This study
Primers		
fucO cloning		
fucO for EcoRI	CGCGCGGAATTCGATTGCCGTAGTGCTGGAGA (SEQ ID NO: 3)	This study
fucO rev BamHI	CGCGCGGGATCCTGCGGTTGGTACGGTAACGG (SEQ ID NO: 4)	This study
ldhA and *ldhL* integration		
ldhA for	GATAACGGAGATCGGGAATG (SEQ ID NO: 5) (for construction of pLOI4652)	(40)
ldhA rev	CTTTGGCTGTCAGTTCACCA (SEQ ID NO: 6) (for construction of pLOI4652)	(40)
ldhA-1	TCTGGAAAAAGGCGAAACCT (SEQ ID NO: 7) (for construction of pLOI4653)	(40)
ldhA-2	TTTGTGCTATAAACGGCGAGT (SEQ ID NO: 8) (for construction of pLOI4653)	(40)
ldhL ORF up	ATGTCTAATATTCAAAATCATCAAAAAGTTGTCCTCGTCG (SEQ ID NO: 9) (for construction of pLOI5174)	This study
ldhL ORF down	TTATTTGTCTTGTTTTTCAGCAAGAGCGTTTAGAC (SEQ ID NO: 10) (for construction of pLOI5174)	This study
ldhA rev1	AAGACTTTCTCCAGTGATGTTGAATCAC (SEQ ID NO: 11) (for construction of pLOI 5174)	This study
ldhA for1	TCTTGCCGCTCCCCT (SEQ ID NO: 12) (for construction of pLOI5174)	This study
Deletion of (*rrlE::[pdc adhA adhB* FRT*]*)		
pdc for	TGGTCTCAAGCATCACTTCG (SEQ ID NO: 13)	This study
adhB rev	TTGGTCAGAGCACAAGCATC (SEQ ID NO: 14)	This study
adhB-1	CCCACGCATTTGAAGCTTAT (SEQ ID NO: 15)	This study
pdc-2	ATCGATTTTAGCCGGAGCTT (SEQ ID NO: 16)	This study
Deletion of (*Zm frg* $estZ_{Pp}$ FRT)		
estZ for	ACTGGCATCTGAGTTCTCTG (SEQ ID NO: 17)	This study
estZ rev	TTCCATGGCGTGAGTTACTG (SEQ ID NO: 18)	This study
estZ-1	CAGACCGTGCGGAATATGGA (SEQ ID NO: 19)	This study
estZ-2	CAGCCTCGATTCGCATGACA (SEQ ID NO: 20)	This study
adhE for	CAATACGCCTTTTGACAGCA (SEQ ID NO: 21)	This study
adhE rev	GCCATCAATGGCAAAAAGTT (SEQ ID NO: 22)	This study
adhE-1	TCAGTAGCGCTGTCTGGCA (SEQ ID NO: 23)	This study
adhE-2	AATGCTCTCCTGATAATGTTAAACTTTTTTAGTA (SEQ ID NO: 24)	This study

Table 1: Bacterial strains, plasmids, and primers

Amplification and sequencing of *yqhD* region		
yqhD for	TATGATGCCAGGCTCGTACA (SEQ ID NO: 25)	This study
yqhD rev	GATCATGCCTTTCCATGCTT (SEQ ID NO: 26)	This study

Table 2: Bacterial strains, plasmids, and primers

Strains, plasmids, or primers	**Relevant characteristics**	**Reference of source**
Stains		
LY180	Δ*frdBC::(Zm frg celY*$_{Ec}$), Δ*ldhA::(Zm* frg *casAB*$_{Ko}$*)*, *adhE::(Zm* frg *estZ*$_{Pp}$ FRT), Δ*ackA*::FRT, *rrlE::(pdc adhA adhB* FRT), Δ*mgsA*::FRT	(29)
EM322	LY180 Δ*yqhD*::FRT	(29)
MM160	Hydrolysate resistant derivative of LY180, *yqhD* (TGG [Trp246] to TAG [*Stop*])	(13), this study
KJ122[a]	Δ*ldhA*, Δ*adhE*, Δ*(focA-pflB)*, Δ*ackA*, Δ*mgsA*, Δ*poxB*, Δ*sfcA*, Δ*aspC*, Δ*citF*, Δ*tdcDE*	(17, 40, 42)
XW055	KJ122 after serial transfer with xylose for succinate production	This study
XW056	XW055 Δ*yqhD*	This study
XW057	XW055 *adhE::fucO*	This study
XW081	XW057 Δ*yqhD*	This study
Plasmids		
pCR2.1	TOPO cloning vector	Invitrogen
pLOI4162	PacI *flanked cat-sacB cassette*	(17)
Deletion of *yqhD*		
pLOI5203	*yqhD* ORF and its adjacent regions cloned into pCR2.1 TOPO vector	This study
pLOI5204	*cat-sacB* cassette cloned into *yqhD* in pLOI5203	This study
pLOI5205	PacI digestion of pLOI5204; self-ligated to delete *yqhD*	This study
adhE::fucO integration		
pLOI5167	*E. coli adhE* ORF and its adjacent regions cloned into pCR2.1 TOPO vector	This study
pLOI5168	*cat-sacB* cassette cloned into *adhE* in pLOI5167	This study
pLOI5209	*adhE* ORF was exactly replaced by *fucO* ORF in pLOI5167	This study
Primers		
Deletion of *yqhD*		
yqhD for	TATGATGCCAGGCTCGTACA (SEQ ID NO: 25)	This study
yqhD rev	GATCATGCCTTTCCATGCTT (SEQ ID NO: 26)	This study

Table 2: Bacterial strains, plasmids, and primers

yqhD-1	GCTTTTTACGCCTCAAACTTTCGT (SEQ ID NO: 27)	This study
yqhD-2	TACTTGCTCCCTTTGCTGG (SEQ ID NO: 28)	This study
adhE::fucO integration		
adhE for	CAATACGCCTTTTGACAGCA (SEQ ID NO: 21)	This study
adhE rev	GCCATCAATGGCAAAAAGTT (SEQ ID NO: 22)	This study
adhE-1	TCAGTAGCGCTGTCTGGCA (SEQ ID NO: 23)	This study
adhE-2	AATGCTCTCCTGATAATGTTAAACTTTTTTAGTA (SEQ ID NO: 24)	This study
adhE-fucO ORF for	AATGCTCTCCTGATAATGTTAAACTTTTTTAGTAATGATGGCTAACAGAATGATTCTGAAC (SEQ ID NO: 29)	This study
adhE-fucO ORF rev	TGCCAGACAGCGCTACTGATTACCAGGCGGTATGGTAAAG (SEQ ID NO: 30)	This study
adhE-fucO-1	CTTTACCATACCGCCTGGTAATCAGTAGCGCTGTCTGGCA (SEQ ID NO: 31)	This study
adhE-fucO-2	GTTCAGAATCATTCTGTTAGCCATCATTACTAAAAAAGTTTAACATTATCAGGAGAGCATT (SEQ ID NO: 32)	This study

[a] The precursor of strain KJ122 also contains spontaneous mutations in *pck*, *ptsI*, and affecting *galP* that were acquired during selection for improvements in growth (17, 40, 42).

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CLAIMS

We claim:

1. An isolated bacterial, fungal or yeast cell having increased NADH-dependent propanediol oxidoreductase (FucO) activity as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF).

2. The isolated bacterial, fungal or yeast cell of claim 1, wherein said bacterial, fungal or yeast cell produces a desired product, or is genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

3. The isolated bacterial, fungal or yeast cell of claims 1-2, wherein said bacterial, fungal or yeast cell exhibits increased production of said desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

4. The isolated bacterial cell of claims 1-3, wherein:

a) expression of the yqhD gene is reduced in said bacterial cell as compared to a reference bacterial cell;

b) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

c) expression of the yqhD gene, yqhC gene and/or the dkgA gene are reduced in said bacterial cell as compared to expression in a reference bacterial cell;

d) expression of the yqhC gene is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

e) expression of the yqhD gene, the yqhC gene and the dkgA genes is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

f) the yqhD gene is not expressed or is deleted in said bacterial cell;

g) the yqhD gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

h) the yqhC gene or yqhD gene, the yqhC gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

i) the yqhC gene is not expressed or is deleted in said bacterial cell;

j) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

k) the dkgA gene is not expressed in said bacterial cell;

l) the activity of the yqhD gene, yqhC gene and/or the dkgA gene product(s) are reduced in said bacterial cell as compared to expression in a reference bacterial cell; or

m) the activity of the yqhC gene product is reduced in said bacterial cell as compared to expression in a reference bacterial cell.

5. The isolated bacterial cell of claim 4, wherein the activity of YqhD protein is reduced in said bacterial cell as compared to a reference bacterial cell.

6. The isolated bacterial cell of claim 4, wherein the activity of the YqhD protein and the activity of the DkgA protein is reduced in said bacterial cell as compared to a reference bacterial cell.

7. The isolated bacterial cell of claim 4, wherein the activity of the YqhC protein is reduced in said bacterial cell as compared to a reference bacterial cell.

8. The isolated bacterial cell of claim 4, wherein regulation of the expression of the yqhD gene is altered to reduce yqhD expression as compared to a reference bacterial cell.

9. The isolated bacterial cell of claim 4, wherein regulation of the expression of the yqhD gene and regulation of expression of the dkgA gene is altered to reduce yqhD and dkgA expression in said bacterial cell as compared to expression in a reference bacterial cell.

10. The isolated bacterial cell of claim 4, wherein regulation of expression of the *yqhC* gene is altered to reduce yqhC expression in said bacterial cell as compared to expression in a reference bacterial cell.

11. The isolated bacterial cell of claim 4, wherein the *yqhC gene, yqhD gene, dkgA* gene or any combination thereof is/are deleted in said bacterial cell.

12. The isolated bacterial cell of claim 4, wherein there is a change in the activity of the yqhD gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

13. The isolated bacterial cell of claim 4, wherein there is a change in the activity of the dkgA gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

14. The isolated bacterial cell of claim 4, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an antisense RNA as compared to a reference bacterial cell.

15. The isolated bacterial cell of claim 4, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an siRNA as compared to a reference bacterial cell.

16. The isolated bacterial, fungal or yeast cell of any preceding claim, wherein FucO activity is increased by:

a) expressing the *FucO* gene in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of the *FucO* gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of the *FucO* gene native promoter with a promoter that increases the level of gene expression in a bacterial cell; or

d) the FucO enzyme is mutated to increase catalytic efficiency or reduce its Km.

17. An isolated bacterial, fungal or yeast cell having increased FucO activity wherein said bacterial, fungal or yeast cell is capable of producing a desired product, or has been genetically engineered to produce a desired product, and wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the

bacterial, fungal or yeast cell capable of producing a desired product, or which has been genetically engineered to produce a desired product, in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, furfural and/or or 5-hydroxymethylfurfural (5-HMF), preferably about 15 mM furfural or about 15 mM to about 30 mM 5-HMF.

18. The isolated bacterial, fungal or yeast cell of claim 17, wherein said cells are grown in the presence of furfural (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM furfural).

19. The isolated bacterial, fungal or yeast cell of claim 17, wherein said cells are grown in the presence of 5-HMF (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM 5-HMF).

20. The isolated bacterial, fungal or yeast cell of claims 17-19, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

21. A method of growing a bacterial, fungal or yeast cell comprising culturing a bacterial, fungal or yeast cell according to any one of claims 1-20 under conditions that allow for the growth of said bacterial, fungal or yeast cell.

22. A method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with the isolated bacterial, fungal or yeast cell according to any one of claims 1-20 and producing said desired product by fermenting said biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source in the presence of said bacterial, fungal or yeast cell.

23. The method of claim 21 or 22, wherein the bacterial, fungal or yeast cell produces a desired product, or has been genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

24. The method according to claim 21, 22 or 23, wherein said bacterial, fungal or yeast cell exhibits increased production of a desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

25. A method of increasing furfural and/or 5-hydroxymethylfurfural (5-HMF) resistance in a bacterial, fungal or yeast cell comprising increasing NADH-dependent propanediol oxidoreductase (FucO) activity in said bacterial, fungal or yeast cell, as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF), wherein said cell has, optionally, been genetically engineered to produce a desired product.

26. The method according to claim 25, wherein:

a) expression of the yqhD gene is reduced in said bacterial cell as compared to a reference bacterial cell;

b) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

c) expression of the yqhD gene, yqhC gene and/or the dkgA gene are reduced in said bacterial cell as compared to expression in a reference bacterial cell;

d) expression of the yqhC gene is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

e) expression of the yqhD gene, the yqhC gene and the dkgA genes is reduced in said bacterial cell as compared to expression in a reference bacterial cell;

f) the yqhD gene is not expressed or is deleted in said bacterial cell;

g) the yqhD gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

h) the yqhC gene or yqhD gene, the yqhC gene and the dkgA gene are not expressed or are deleted in said bacterial cell;

i) the yqhC gene is not expressed or is deleted in said bacterial cell;

j) expression of the dkgA gene is reduced in said bacterial cell as compared to a reference bacterial cell;

k) the dkgA gene is not expressed in said bacterial cell;

l) the activity of the yqhD gene, yqhC gene and/or the dkgA gene product(s) are reduced in said bacterial cell as compared to expression in a reference bacterial cell; or

m) the activity of the yqhC gene product is reduced in said bacterial cell as compared to expression in a reference bacterial cell.

27. The method according to claim 26, wherein the activity of YqhD protein is reduced in said bacterial cell as compared to a reference bacterial cell.

28. The method according to claim 26, wherein the activity of the YqhD protein and the activity of the DkgA protein in said bacterial cell is reduced in said bacterial cell as compared to a reference bacterial cell.

29. The method according to claim 26, wherein the activity of the YqhC protein is reduced in said bacterial cell as compared to a reference bacterial cell.

30. The method according to claim 26, wherein regulation of the expression of the yqhD gene is altered to reduce yqhD expression in said bacterial cell as compared to a reference bacterial cell.

31. The method according to claim 26, wherein regulation of the expression of the yqhD gene and regulation of expression of the dkgA gene is altered to reduce yqhD and dkgA expression in said bacterial cell as compared to expression in a reference bacterial cell.

32. The method according to claim 26, wherein regulation of expression of the *yqhC* gene is altered to reduce yqhC expression in said bacterial cell as compared to expression in a reference bacterial cell.

33. The method according to claim 26, wherein the *yqhC gene, yqhD gene, dkgA* gene or any combination thereof is/are deleted.

34. The method according to claim 26, wherein there is a change in the activity of the yqhD gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

35. The method according to claim 26, wherein there is a change in the activity of the dkgA gene promoter or regulatory protein in said bacterial cell as compared to a reference bacterial cell.

36. The method according to claim 26, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an antisense RNA as compared to a reference bacterial cell.

37. The method according to claim 26, wherein the level of YqhD, DkgA and/or YqhC protein is reduced in said bacterial cell due to the addition of an siRNA as compared to a reference bacterial cell.

38. The method according to any one of claims 25-37, wherein FucO activity is increased by:

a) expressing the *FucO* gene in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of the *FucO* gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of the *FucO* gene native promoter with a promoter that increases the level of gene expression in a bacterial, fungal or yeast cell; or

d) the FucO enzyme is mutated to increase catalytic efficiency or reduce its Km.

39. The method according to claim 25, wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the bacterial, fungal or yeast cell in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product in the presence of furfural and/or or 5-

hydroxymethylfurfural (5-HMF), wherein said candidate mutant strain has been genetically engineered to produce a desired product.

40. The method according to claim 39, wherein said cell is grown in the presence of furfural.

41. The method according to claim 39, wherein said cell is grown in the presence of 5-HMF.

42. The method according to any one of claims 39-41, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

43. The isolated bacterial cell or method according to any one of claims 1-42, wherein said bacterial cell is a Gram-negative or a Gram-positive bacterial cell.

44. The isolated bacterial cell or method according to claim 43, wherein the Gram-negative bacterial cell is a bacterial cell selected from the genera of *Escherichia, Zymomonas, Acinetobacter, Gluconobacter, Geobacter, Shewanella, Salmonella, Enterobacter* or *Klebsiella* and the Gram-positive bacteria is a bacterial cell selected from the genera of *Bacillus, Clostridium, Corynebacterial cell, Lactobacillus, Lactococcus, Oenococcus, Streptococcus* and *Eubacterial cell.*

45. The isolated bacterial cell or method according to claim 44, wherein the bacterial cell is *Escherichia coli* or *Klebsiella oxytoca.*

46. The isolated bacterial cell or method according to claim 43, wherein said bacterial cell is selected from *Thermoanaerobes, Bacillus spp.*, *Paenibacillus* spp. or *Geobacillus* spp.

47. The isolated yeast cell or method according to any one of claims 1-42, wherein said yeast cell is a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, or *Yarrowia* cell.

48. The isolated yeast cell or method according to claim 47, wherein said yeast cell is *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis*, or *Yarrowia lipolytica.*

49. The isolated fungal cell or method according to claims 1-42, wherein said fungal cell is a *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes*, or *Trichoderma* cell.

50. The isolated fungal cell or method according to claim 49, wherein said fungal cell is an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei*, or *Trichoderma viride* cell.

51. The isolated bacterial, fungal or yeast cell according to any one of claims 1-20 or 43-50, wherein said bacterial, fungal or yeast cell produce a desired product in the

presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural and/or 5-HMF.

52. An isolated bacterial, fungal or yeast cell having increased NADH-dependent furfural or 5-HMF oxidoreductase activity as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased NADH-dependent furfural or 5-HMF oxidoreductase activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF) and wherein said cell has, optionally, been genetically engineered to produce a desired product.

53. The isolated bacterial, fungal or yeast cell of claim 52, wherein said bacterial cell produces a desired product selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

54. The isolated bacterial, fungal or yeast cell of claims 52-53, wherein said bacterial, fungal or yeast cell exhibits increased production of said desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

55. The isolated bacterial, fungal or yeast cell of claims 52, 53 or 54, wherein FucO activity is increased by:

a) expressing one or more NADH-dependent furfural or 5-HMF oxidoreductase in a plasmid or a multicopy plasmid with a native promoter or a promoter sequence;

b) transposon integration of additional copies of a NADH-dependent furfural or 5-HMF oxidoreductase gene within the chromosome of a bacterial, fungal or yeast cell;

c) replacement of a native promoter for a NADH-dependent furfural or 5-HMF oxidoreductase with a promoter that increases the level of gene expression in a bacterial, fungal or yeast cell; or

d) a NADH-dependent furfural or 5-HMF oxidoreductase enzyme is mutated to increase catalytic efficiency or reduce its Km.

56. An isolated bacterial, fungal or yeast cell having increased FucO activity wherein said bacterial, fungal or yeast cell is capable of producing a desired product and wherein said bacterial, fungal or yeast cell is prepared by a process comprising: a) growing a candidate mutant strain of the bacterial, fungal or yeast cell in the presence of furfural or 5-hydroxymethylfurfural (5-HMF); and b) selecting mutants that produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, furfural and/or or 5-hydroxymethylfurfural (5-HMF), preferably about 15 mM furfural or about 15 mM to about 30 mM 5-HMF, wherein said candidate mutant had optionally been genetically engineered to produce a desired product.

57. The isolated bacterial, fungal or yeast cell of claim 56, wherein said cells are grown in the presence of furfural (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM furfural).

58. The isolated bacterial, fungal or yeast cell of claim 56, wherein said cells are grown in the presence of 5-HMF (about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 to about 30 mM, about 15 mM or about 15 mM to about 30 mM 5-HMF).

59. The isolated bacterial, fungal or yeast cell of claims 56-58, wherein the selected mutants are compared to a reference bacterial, fungal or yeast cell for the ability to produce a desired product in the presence of furfural and/or or 5-hydroxymethylfurfural (5-HMF).

60. A method of growing a bacterial, fungal or yeast cell comprising culturing a bacterial, fungal or yeast cell according to any one of claims 52-59 under conditions that allow for the growth of said bacterial, fungal or yeast cell.

61. A method for producing a desired product from a biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source comprising contacting the biomass, hemicellulosic biomass, lignocellulosic biomass, cellulosic biomass or oligosaccharide with the isolated bacterial, fungal or yeast cell according to any one of claims 52-59 and producing said desired product by fermenting said

biomass, a hemicellulosic biomass, a lignocellulosic biomass, a cellulosic biomass or an oligosaccharide source in the presence of said bacterial, fungal or yeast cell.

62. The method of claim 60 or 61, wherein the bacterial, fungal or yeast cell produces a desired product selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

63. The method according to claim 60, 61 or 62, wherein said bacterial, fungal or yeast cell exhibits increased production of a desired product as compared to a reference bacterial, fungal or yeast cell in the presence of furfural and/or 5-hydroxymethylfurfural (5-HMF).

64. A method of increasing furfural and/or 5-hydroxymethylfurfural (5-HMF) resistance in a bacterial, fungal or yeast cell comprising increasing NADH-dependent furfural or 5-HMF oxidoreductase activity in said bacterial, fungal or yeast cell, as compared to a reference bacterial, fungal or yeast cell, wherein said bacterial, fungal or yeast cell having increased FucO activity reduces furfural and/or 5-hydroxymethylfurfural (5-HMF) and has been, optionally, genetically engineered to produce a desired product.

65. The isolated bacterial cell or method according to any one of claims 52-64, wherein said bacterial cell is a Gram-negative or a Gram-positive bacterial cell.

66. The isolated bacterial cell or method according to claim 65, wherein the Gram-negative bacterial cell is a bacterial cell selected from the genera of *Escherichia, Zymomonas, Acinetobacter, Gluconobacter, Geobacter, Shewanella, Salmonella, Enterobacter* or *Klebsiella* and the Gram-positive bacteria is a bacterial cell selected from the genera of *Bacillus, Clostridium, Corynebacterial cell, Lactobacillus, Lactococcus, Oenococcus, Streptococcus* and *Eubacterial cell.*

67. The isolated bacterial cell or method according to claim 66, wherein the bacterial cell is *Escherichia coli* or *Klebsiella oxytoca.*

68. The isolated bacterial cell or method according to claim 66, wherein said bacterial cell is selected from *Thermoanaerobes, Bacillus spp.*, *Paenibacillus* spp. or *Geobacillus* spp.

69. The isolated yeast cell or method according to any one of claims 52-64, wherein said yeast cell is a *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, or *Yarrowia* cell.

70. The isolated yeast cell or method according to claim 69, wherein said yeast cell is *Kluyveromyces lactis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis, Saccharomyces oviformis,* or *Yarrowia lipolytica.*

71. The isolated fungal cell or method according to claims 52-64, wherein said fungal cell is a *Acremonium, Aspergillus, Aureobasidium, Bjerkandera, Ceriporiopsis, Chrysosporium, Coprinus, Coriolus, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Phlebia, Piromyces, Pleurotus, Schizophyllum, Talaromyces, Thermoascus, Thielavia, Tolypocladium, Trametes*, or *Trichoderma* cell.

72. The isolated fungal cell or method according to claim 71, wherein said fungal cell is an *Aspergillus awamori, Aspergillus foetidus, Aspergillus fumigatus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Bjerkandera adusta, Ceriporiopsis aneirina, Ceriporiopsis caregiea, Ceriporiopsis gilvescens, Ceriporiopsis pannocinta, Ceriporiopsis rivulosa, Ceriporiopsis subrufa, Ceriporiopsis subvermispora, Chrysosporium inops, Chrysosporium keratinophilum, Chrysosporium lucknowense, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium queenslandicum, Chrysosporium tropicum, Chrysosporium zonatum, Coprinus cinereus, Coriolus hirsutus, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola*

lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii, Thielavia terrestris, Trametes villosa, Trametes versicolor, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei, or *Trichoderma viride* cell.

73. The isolated bacterial, fungal or yeast cell or method according to any one of claims 52-72, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural and/or 5-HMF.

74. The isolated bacterial, fungal or yeast cell or method according to any one of claims 52-72, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural.

75. The isolated bacterial, fungal or yeast cell or method according to any one of claims 52-72, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM 5-HMF.

76. The method of claims 60, 61, 62 or 63, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM furfural.

77. The method of claims 60, 61, 62 or 63, wherein said bacterial, fungal or yeast cell produce a desired product in the presence of about 5 mM to about 40 mM, about 5 mM to about 20 mM, about 15 mM to about 30 mM, or about 15 mM 5-HMF.

78. The isolated bacterial, fungal or yeast cell or method according to any one of the preceding claims, wherein said bacterial, fungal or yeast cell has been genetically engineered to produce a desired product, selected from the group consisting of ethanol, lactic acid, succinic acid, malic acid, acetic acid, 1,3-propanediol, 2,3-propanediol, 1,4-butanediol, 2,3-butanediol, butanol, pyruvate, dicarboxylic acids, adipic acid and amino acids.

Fig. 1

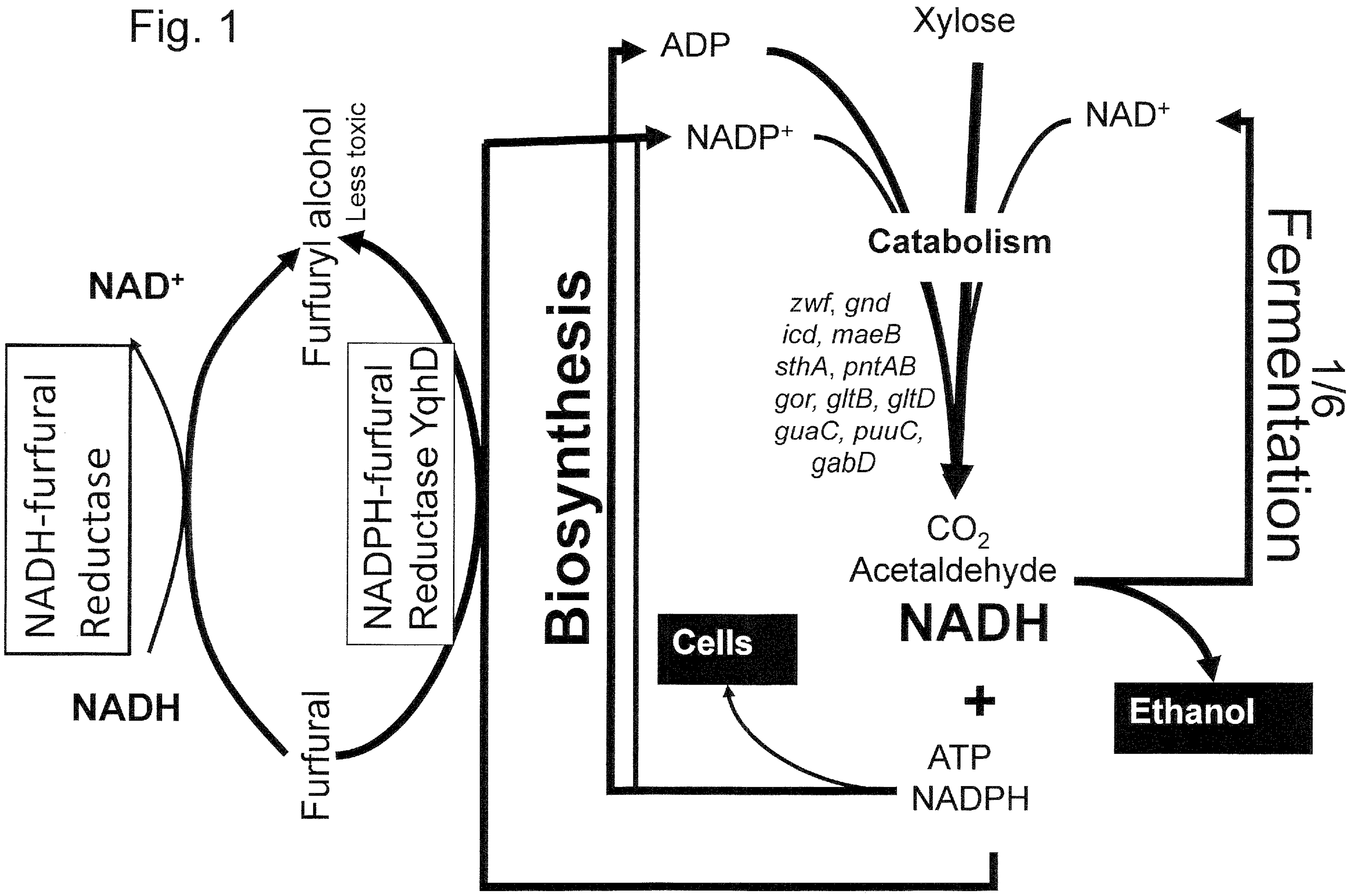

Xylose
ADP
NADP⁺
NAD⁺
Catabolism
zwf, gnd
icd, maeB
sthA, pntAB
gor, gltB, gltD
guaC, puuC,
gabD
CO₂
Acetaldehyde
NADH
+
ATP
NADPH
1/6
Fermentation
Ethanol
Cells
Biosynthesis
Furfuryl alcohol
Less toxic
Furfural
NADPH-furfural
Reductase YqhD
NAD⁺
NADH
NADH-furfural
Reductase

Fig. 2

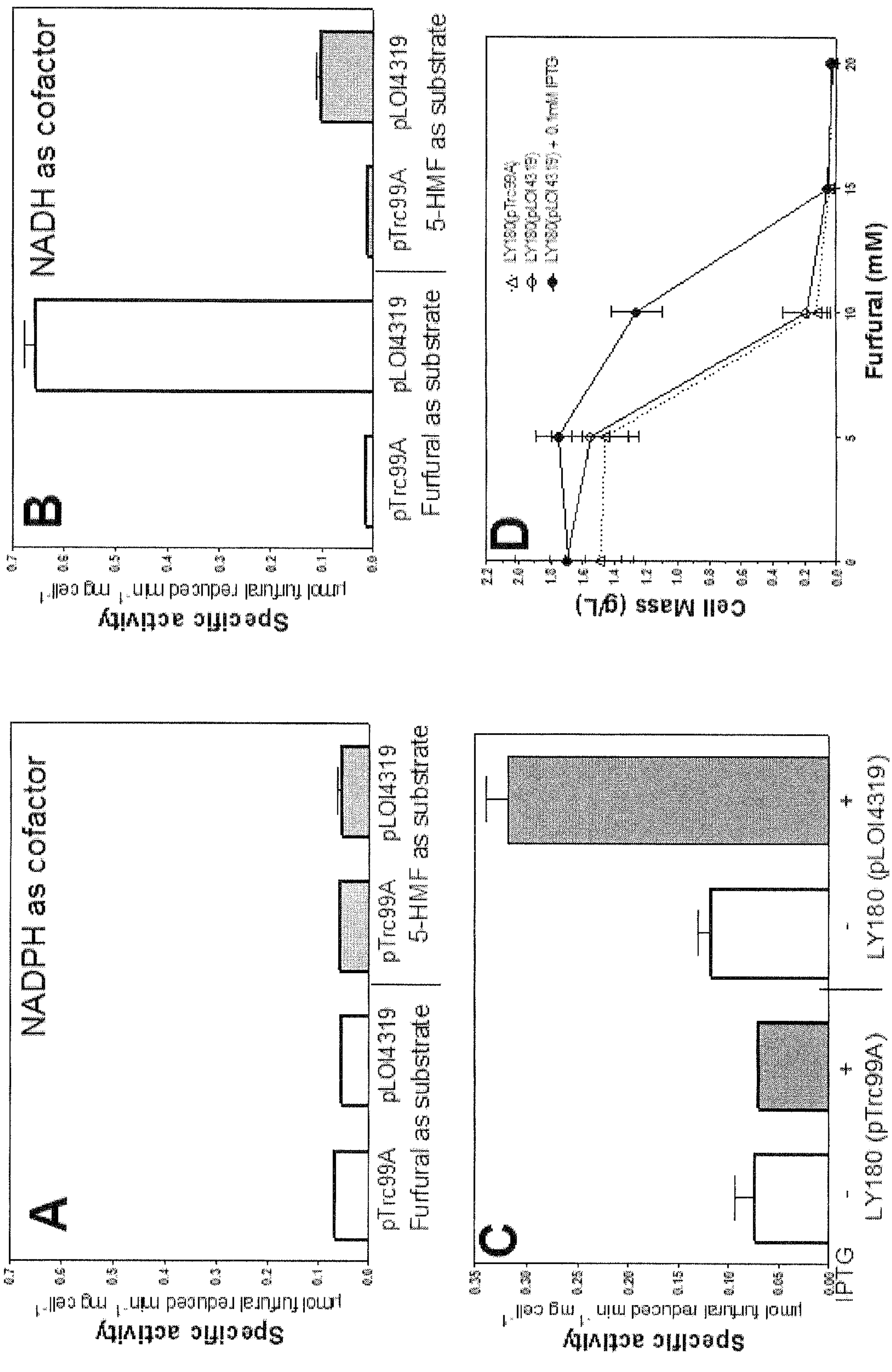
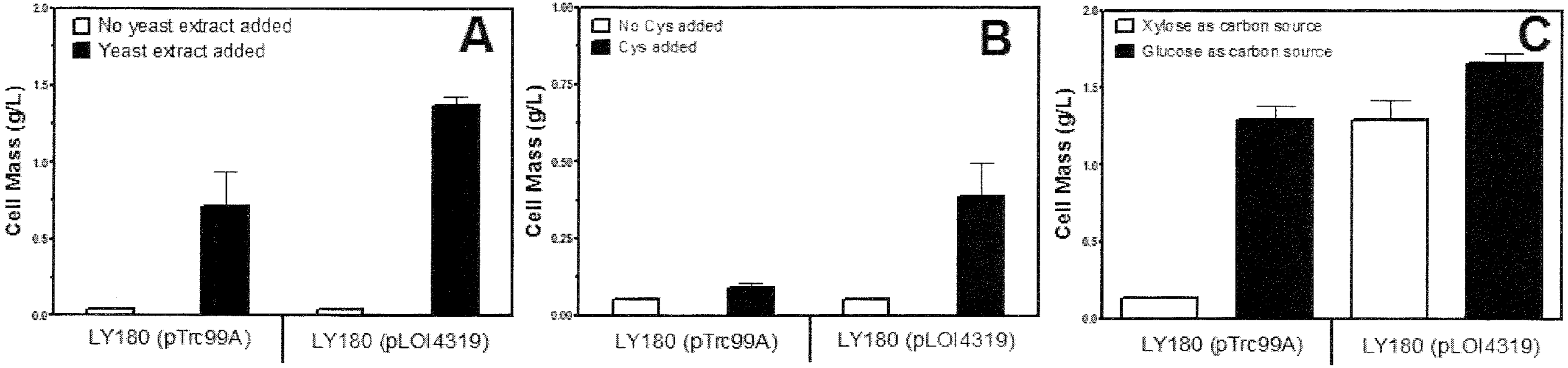

A
NADPH as cofactor
Specific activity
μmol furfural reduced min⁻¹ mg cell⁻¹
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
pTrc99A
pLOI4319
Furfural as substrate
pTrc99A
pLOI4319
5-HMF as substrate
B
NADH as cofactor
Specific activity
μmol furfural reduced min⁻¹ mg cell⁻¹
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
pTrc99A
pLOI4319
Furfural as substrate
pTrc99A
pLOI4319
5-HMF as substrate
C
Specific activity
μmol furfural reduced min⁻¹ mg cell⁻¹
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
IPTG
-
+
-
+
LY180 (pTrc99A)
LY180 (pLOI4319)
D
Cell Mass (g/L)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Furfural (mM)
LY180(pTrc99A)
LY180(pLOI4319)
LY180(pLOI4319) + 0.1mM IPTG

Fig. 3


A
B
C
Cell Mass (g/L)
0.0
0.5
1.0
1.5
2.0
☐ No yeast extract added
■ Yeast extract added
0.00
0.25
0.50
0.75
1.00
☐ No Cys added
■ Cys added
☐ Xylose as carbon source
■ Glucose as carbon source
LY180 (pTrc99A)
LY180 (pLOI4319)

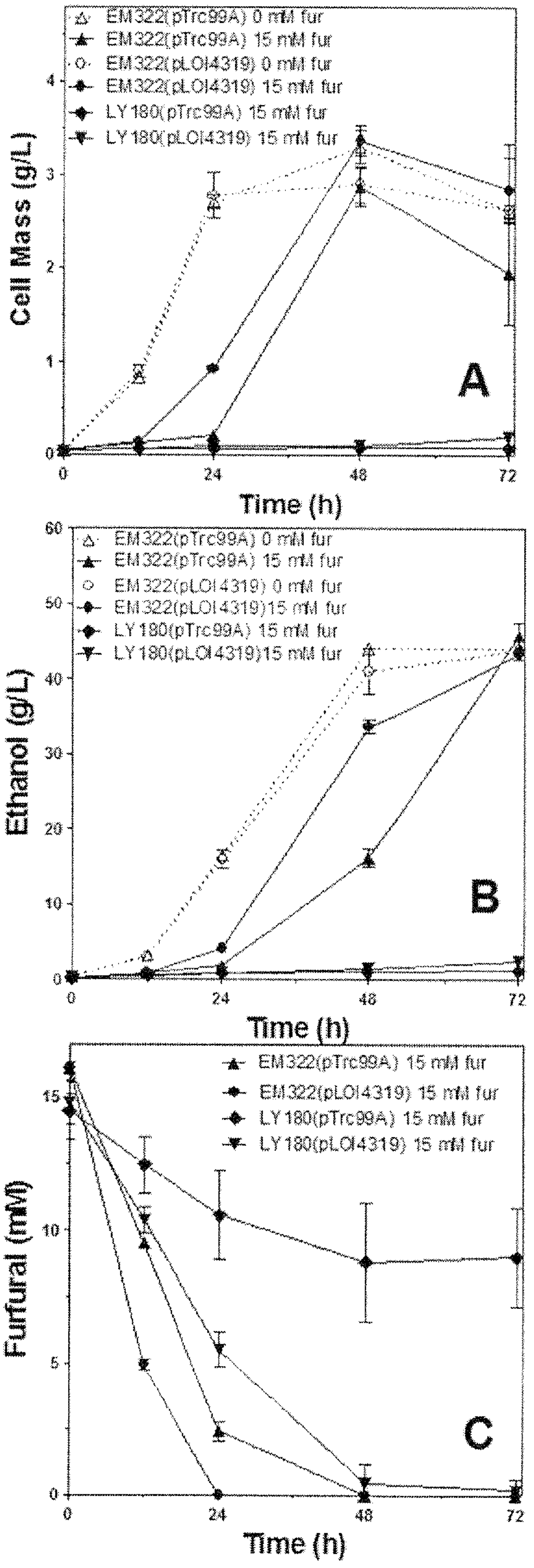

EM322(pTrc99A) 0 mM fur
EM322(pTrc99A) 15 mM fur
EM322(pLOI4319) 0 mM fur
EM322(pLOI4319) 15 mM fur
LY180(pTrc99A) 15 mM fur
LY180(pLOI4319) 15 mM fur
Cell Mass (g/L)
0
1
2
3
4
A
0
24
48
72
Time (h)
EM322(pTrc99A) 0 mM fur
EM322(pTrc99A) 15 mM fur
EM322(pLOI4319) 0 mM fur
EM322(pLOI4319)15 mM fur
LY180(pTrc99A) 15 mM fur
LY180(pLOI4319)15 mM fur
Ethanol (g/L)
0
10
20
30
40
50
60
B
0
24
48
72
Time (h)
EM322(pTrc99A) 15 mM fur
EM322(pLOI4319) 15 mM fur
LY180(pTrc99A) 15 mM fur
LY180(pLOI4319) 15 mM fur
Furfural (mM)
0
5
10
15
C
0
24
48
72
Time (h)

Fig. 4

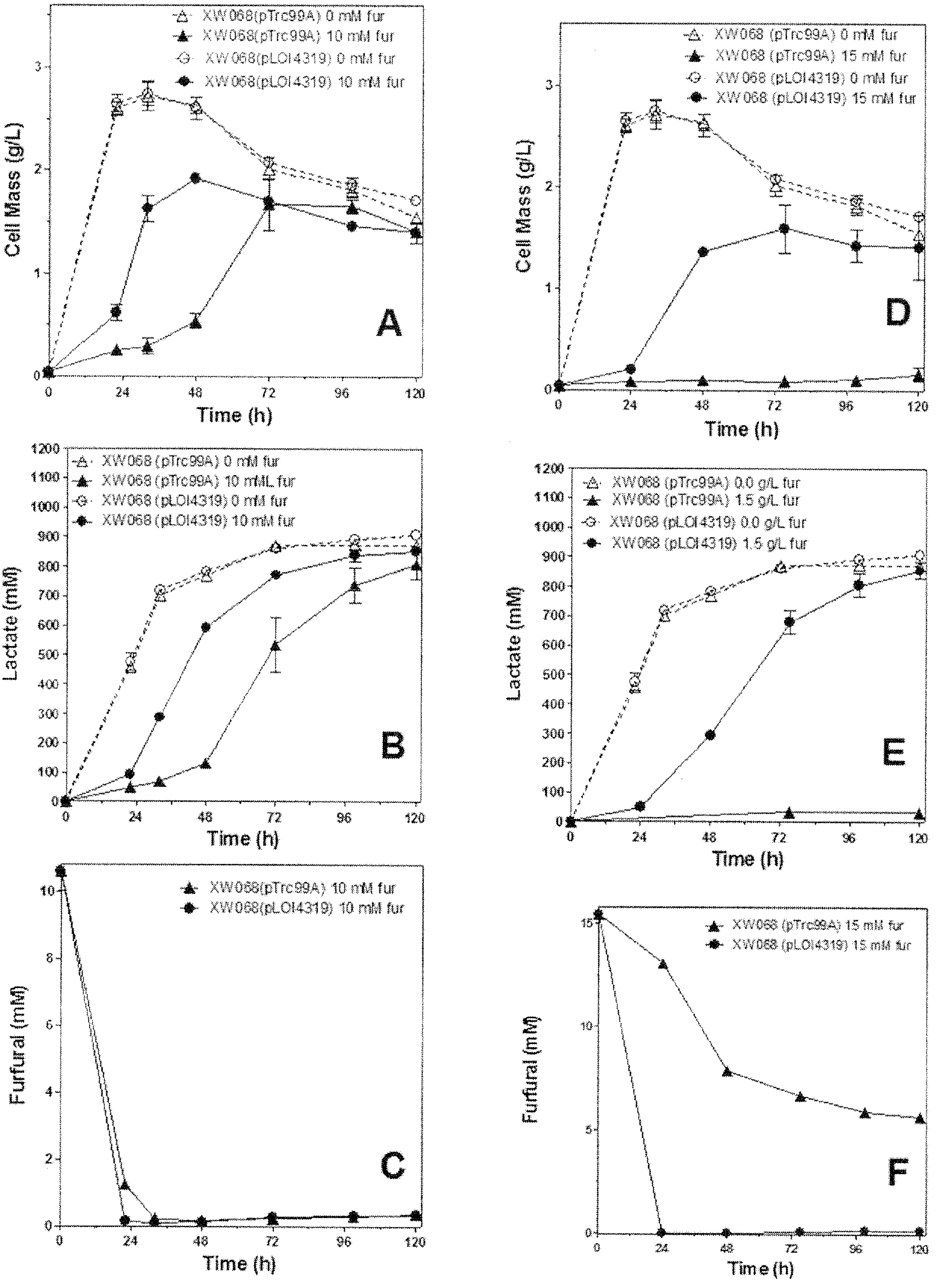

XW068(pTrc99A) 0 mM fur
XW068(pTrc99A) 10 mM fur
XW068(pLOI4319) 0 mM fur
XW068(pLOI4319) 10 mM fur
Cell Mass (g/L)
Time (h)
A
XW068 (pTrc99A) 0 mM fur
XW068 (pTrc99A) 15 mM fur
XW068 (pLOI4319) 0 mM fur
XW068 (pLOI4319) 15 mM fur
Cell Mass (g/L)
Time (h)
D
XW068 (pTrc99A) 0 mM fur
XW068 (pTrc99A) 10 mML fur
XW068 (pLOI4319) 0 mM fur
XW068 (pLOI4319) 10 mM fur
Lactate (mM)
Time (h)
B
XW068 (pTrc99A) 0.0 g/L fur
XW068 (pTrc99A) 1.5 g/L fur
XW068 (pLOI4319) 0.0 g/L fur
XW068 (pLOI4319) 1.5 g/L fur
Lactate (mM)
Time (h)
E
XW068(pTrc99A) 10 mM fur
XW068(pLOI4319) 10 mM fur
Furfural (mM)
Time (h)
C
XW068 (pTrc99A) 15 mM fur
XW068 (pLOI4319) 15 mM fur
Furfural (mM)
Time (h)
F
0
24
48
72
96
120

Fig. 5

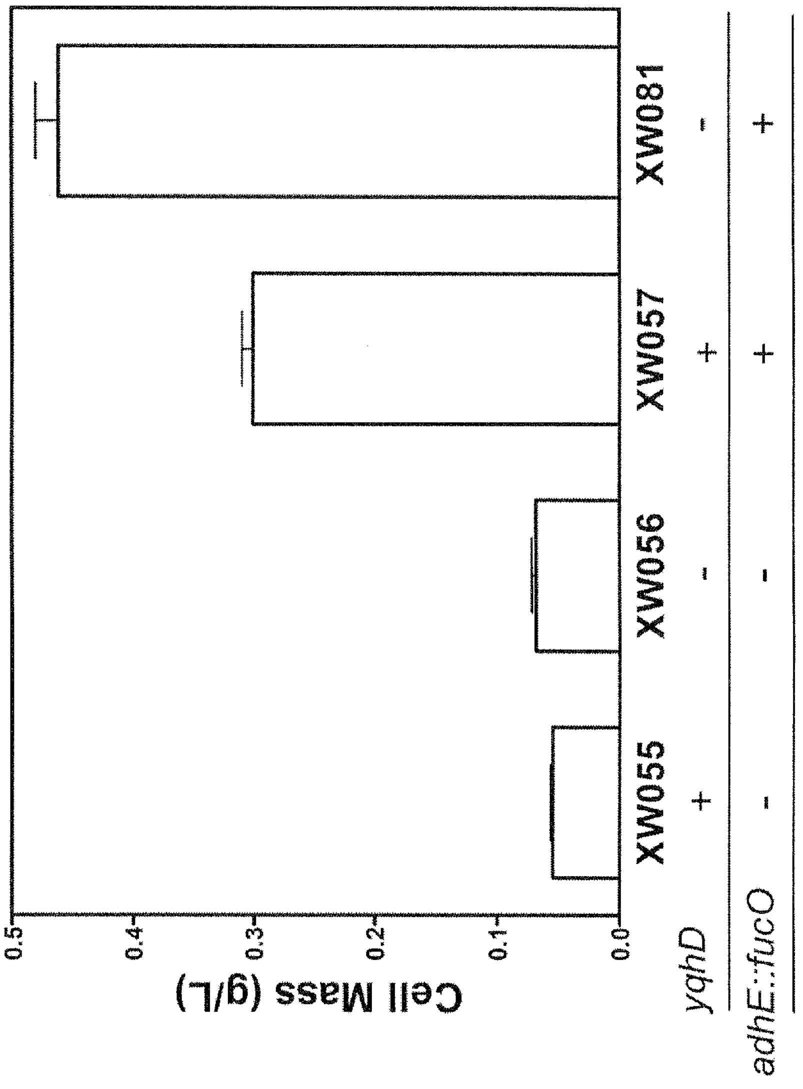

Cell Mass (g/L)
0.0
0.1
0.2
0.3
0.4
0.5
XW055
XW056
XW057
XW081
yqhD
+
-
+
-
adhE::fucO
-
-
+
+

Fig. 6