



US 20250263756A1

(19) United States

(12) Patent Application Publication

Broadbent et al.

(10) Pub. No.: US 2025/0263756 A1

(43) Pub. Date: Aug. 21, 2025

(54) SYNERGISTIC BACTERIAL AND YEAST COMBINATIONS

(71) Applicant: Lallemand Hungary Liquidity Management LLC, Budapest (HU)

(72) Inventors: Jeffery R. Broadbent, Amalga, UT (US); Aaron Argyros, Lebanon, NH (US); Brooks Henningsen, Salisbury, NH (US); Fernanda Cristina Firmino, Atlanta, GA (US); Ekkarat Phrommao, Lebanon, NH (US); James L. Steele, Lebanon, NH (US)

(21) Appl. No.: 18/967,018

(22) Filed: Dec. 3, 2024

Related U.S. Application Data

(63) Continuation of application No. 17/292,358, filed on May 7, 2021, filed as application No. PCT/IB2019/059765 on Nov. 13, 2019, now abandoned.

(60) Provisional application No. 62/760,472, filed on Nov. 13, 2018.

Publication Classification

(51) Int. Cl.

CI2P 7/14	(2006.01)
CI2N 9/88	(2006.01)
CI2P 7/10	(2006.01)
CI2P 7/54	(2006.01)
CI2P 19/02	(2006.01)

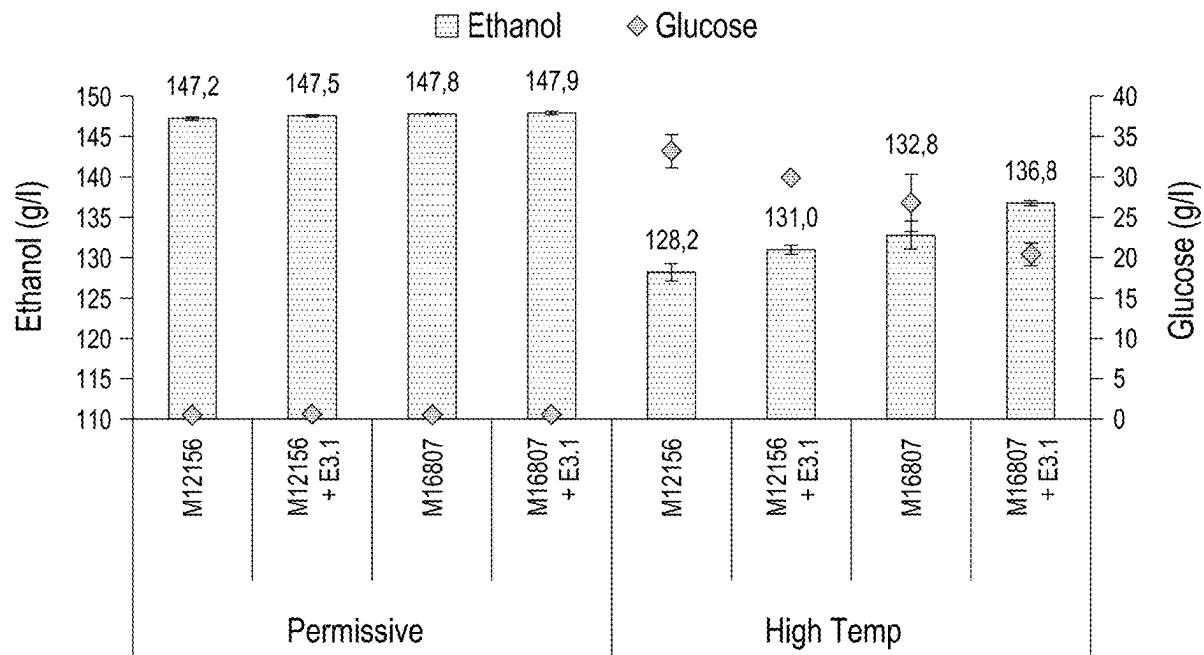
(52) U.S. Cl.

CPC	CI2P 7/14 (2013.01); CI2N 9/88 (2013.01); CI2P 7/10 (2013.01); CI2P 7/54 (2013.01); CI2P 19/02 (2013.01)
-----------	--

(57)

ABSTRACT

The present disclosure concerns a symbiotic combination of host cells engineered to produce a first metabolic product, for example a carbohydrate, and to convert the second metabolic product into a second metabolic product, for example an alcohol.

Specification includes a Sequence Listing.

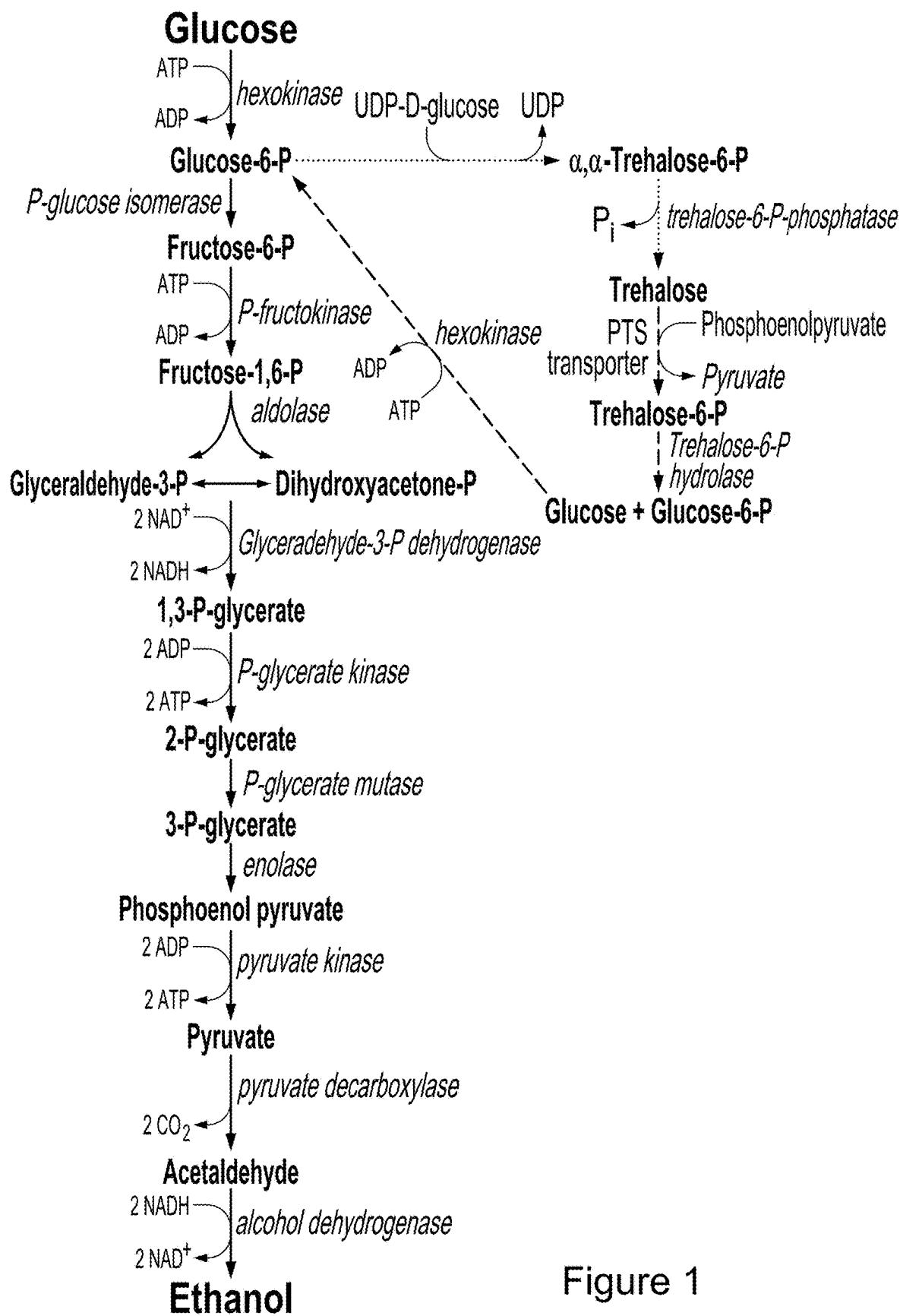


Figure 1

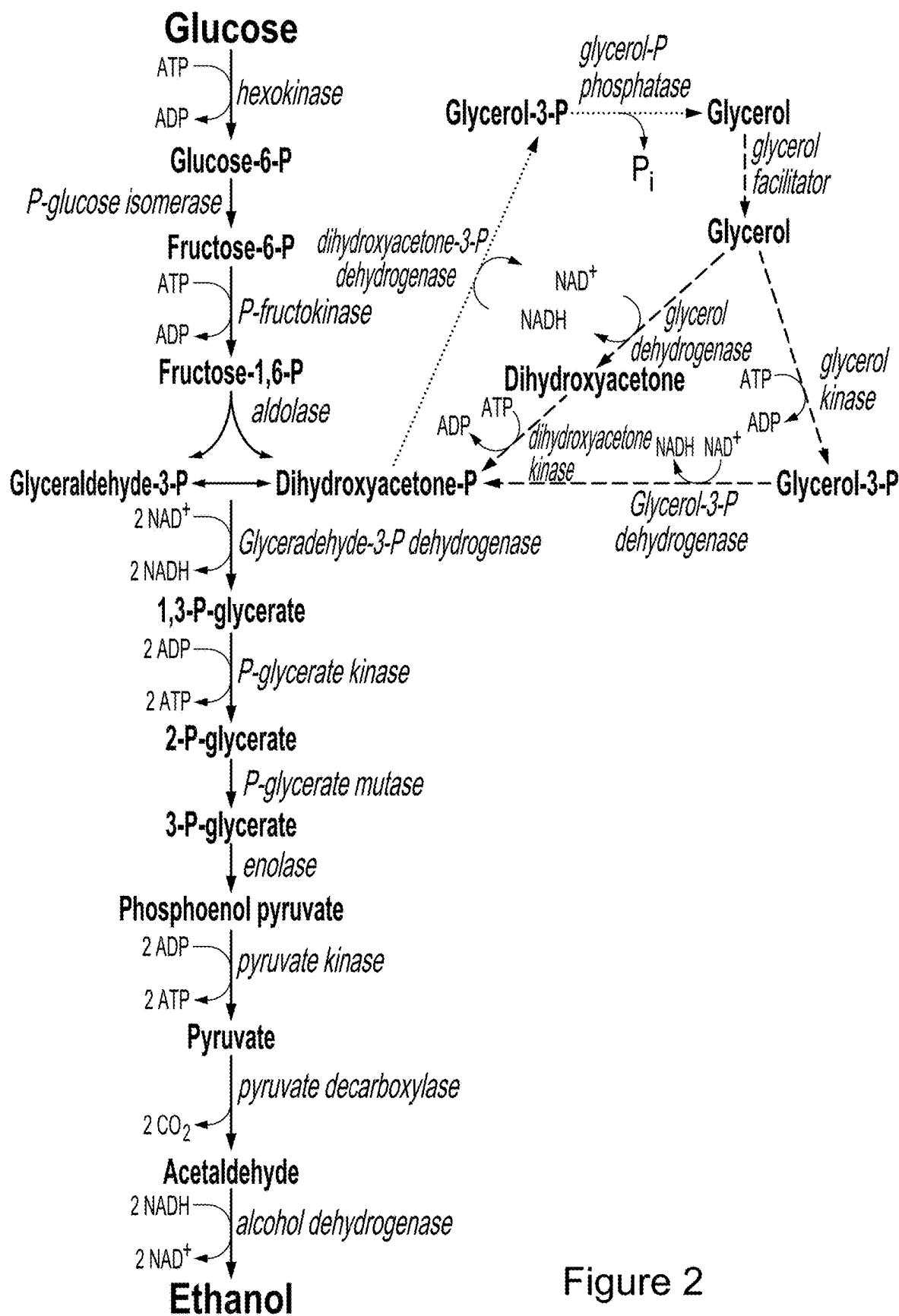


Figure 2

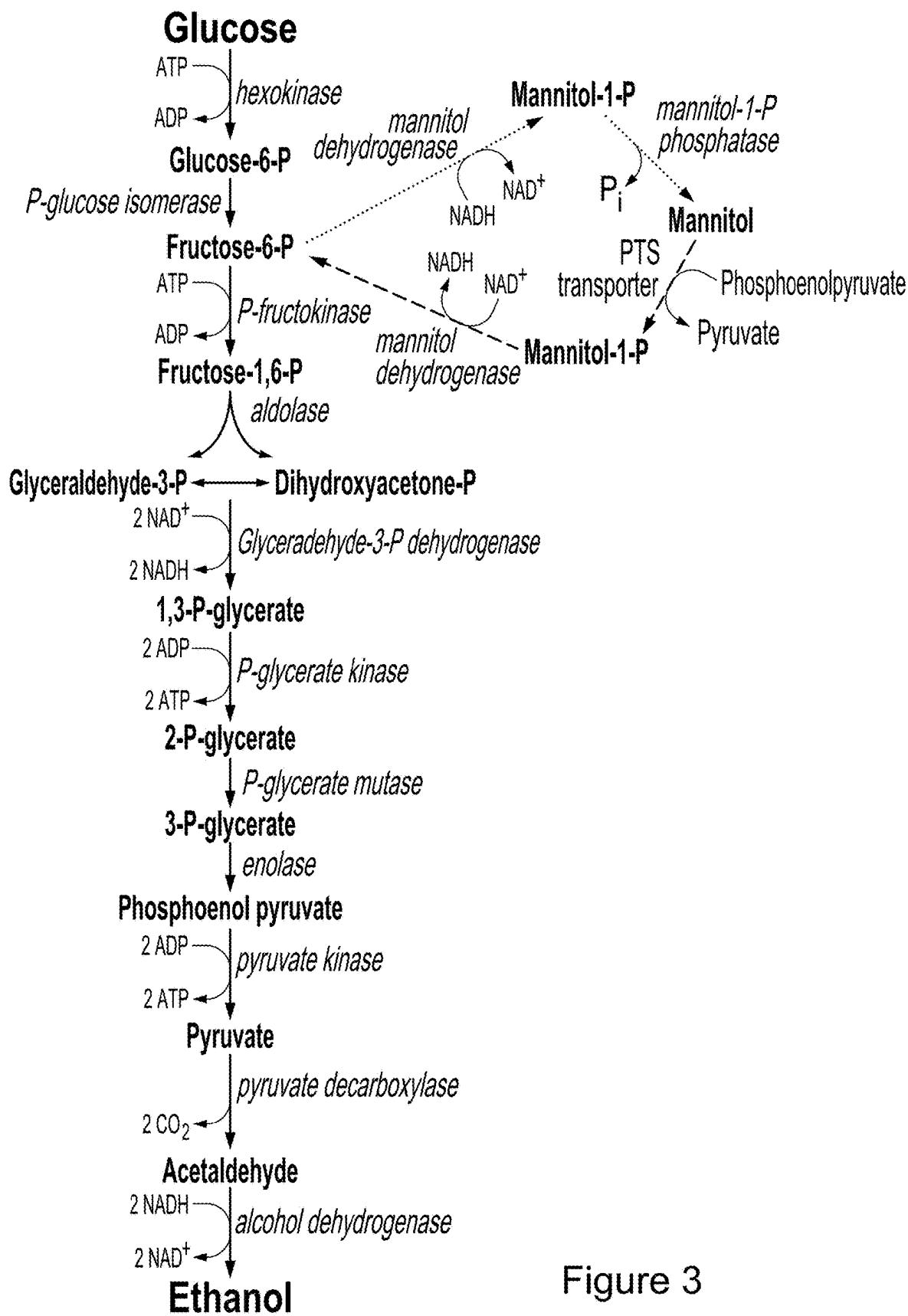


Figure 3

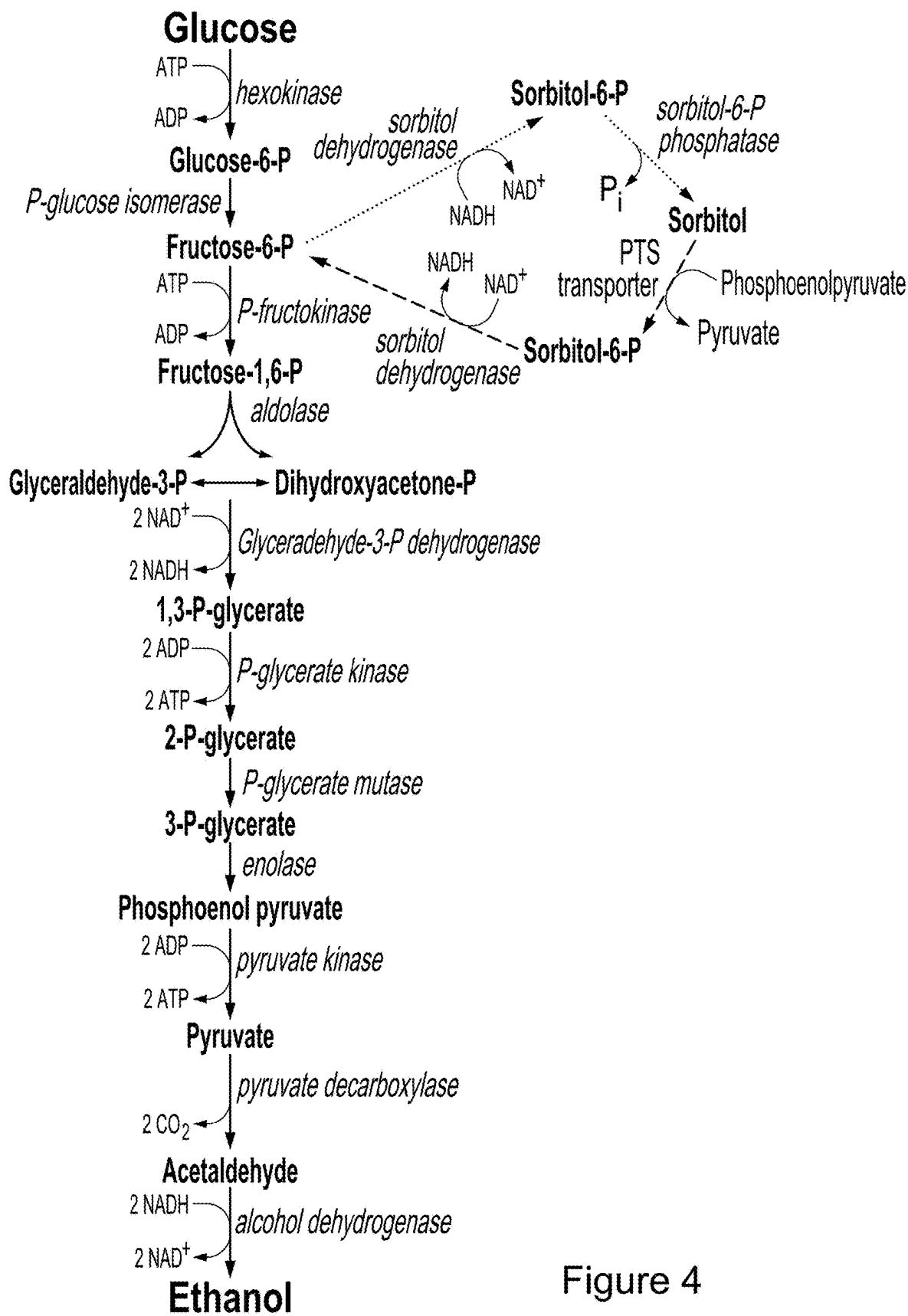


Figure 4

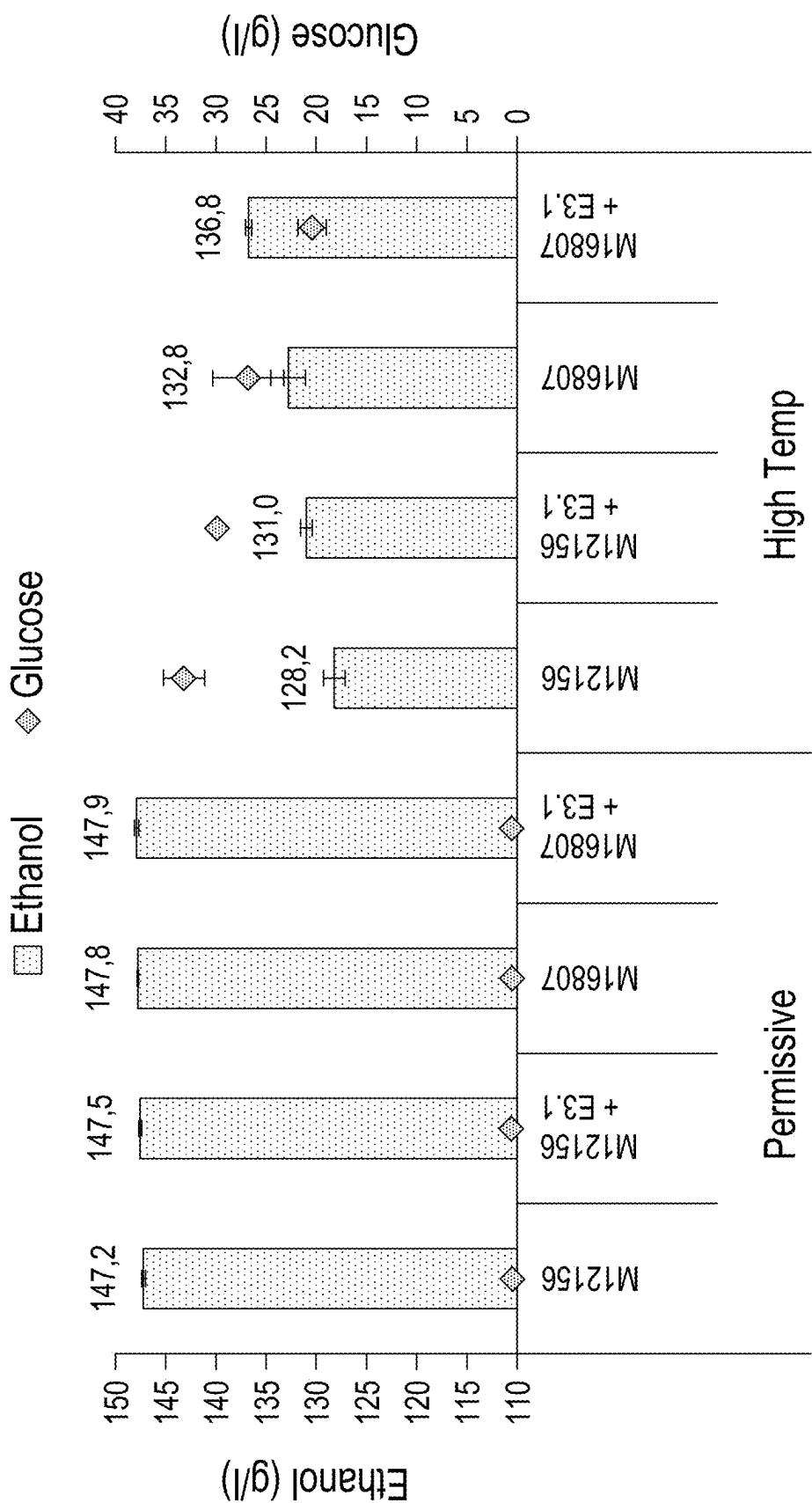


Figure 5

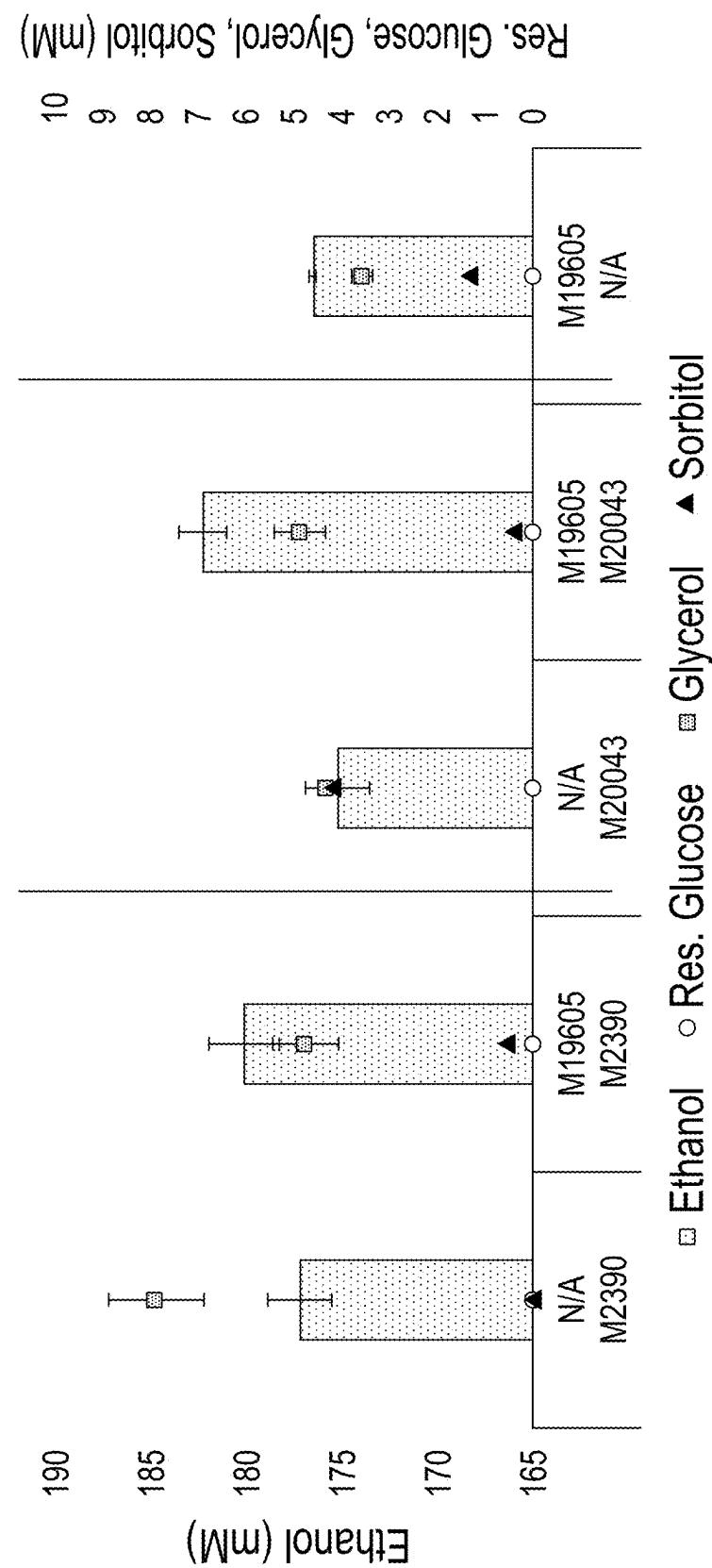


Figure 6

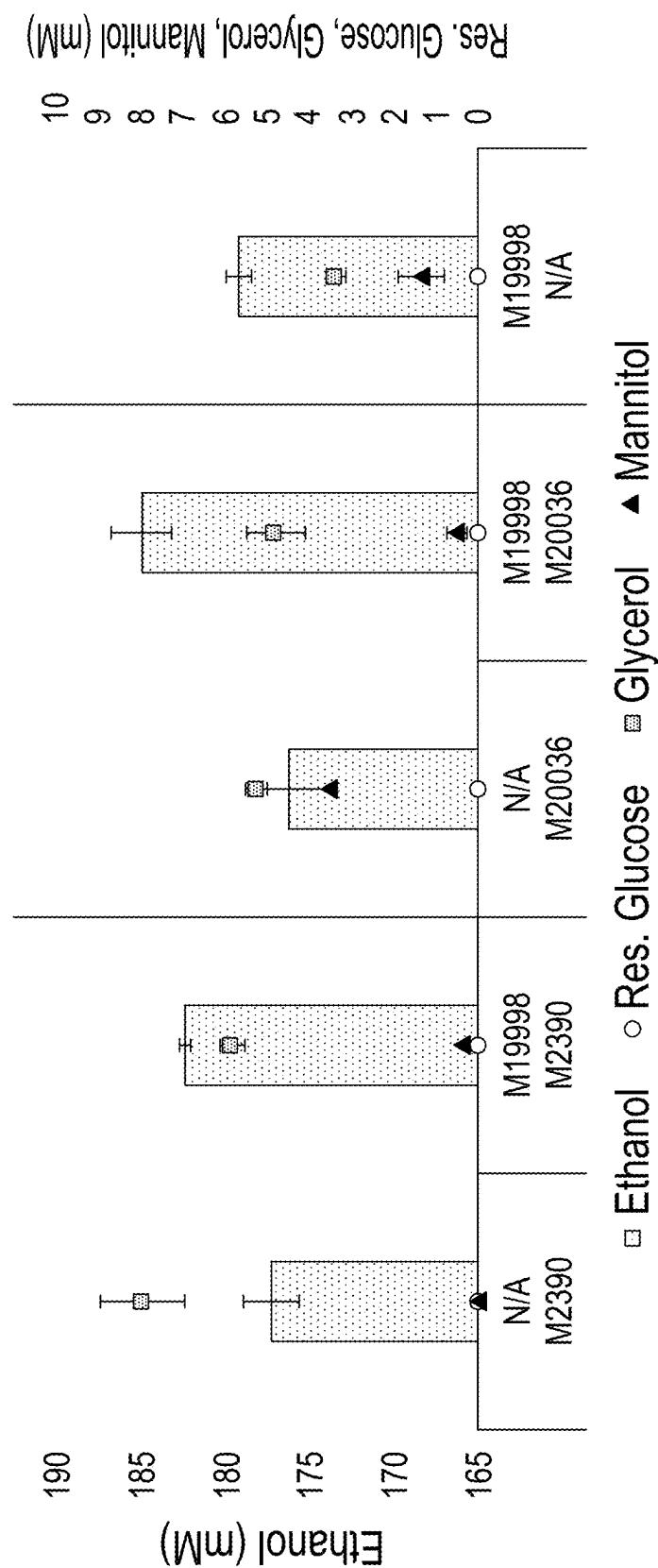


Figure 7

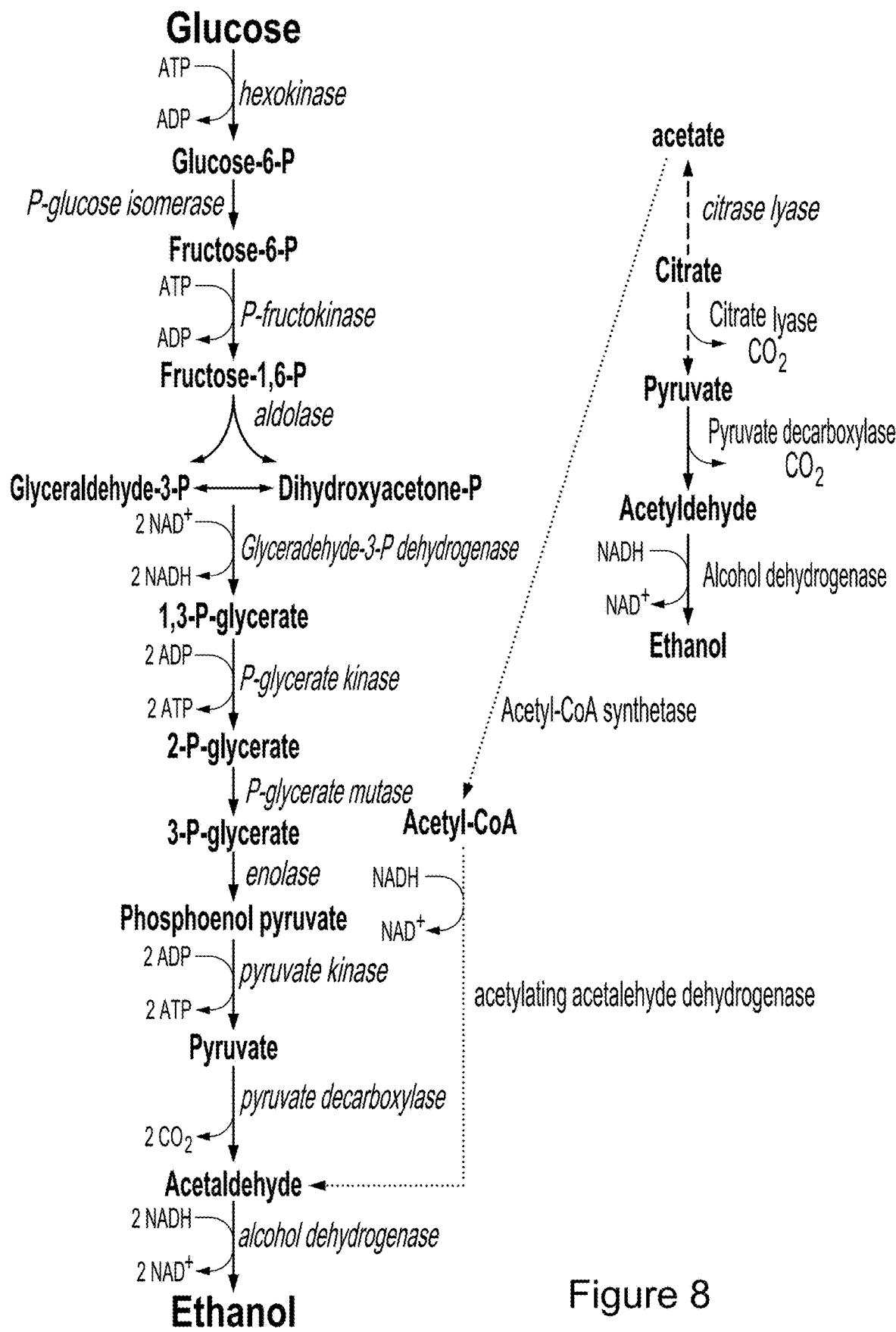


Figure 8

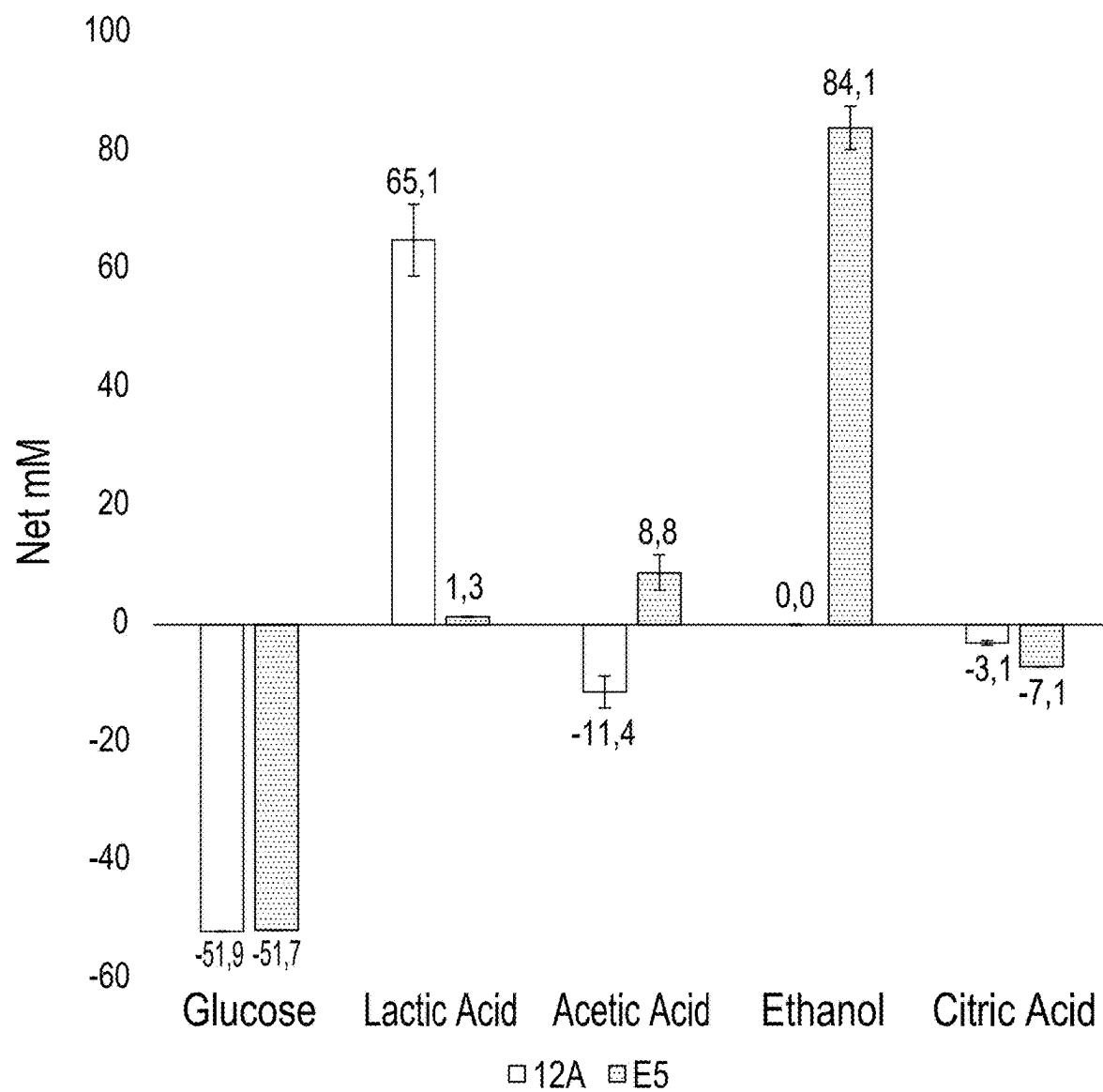


Figure 9

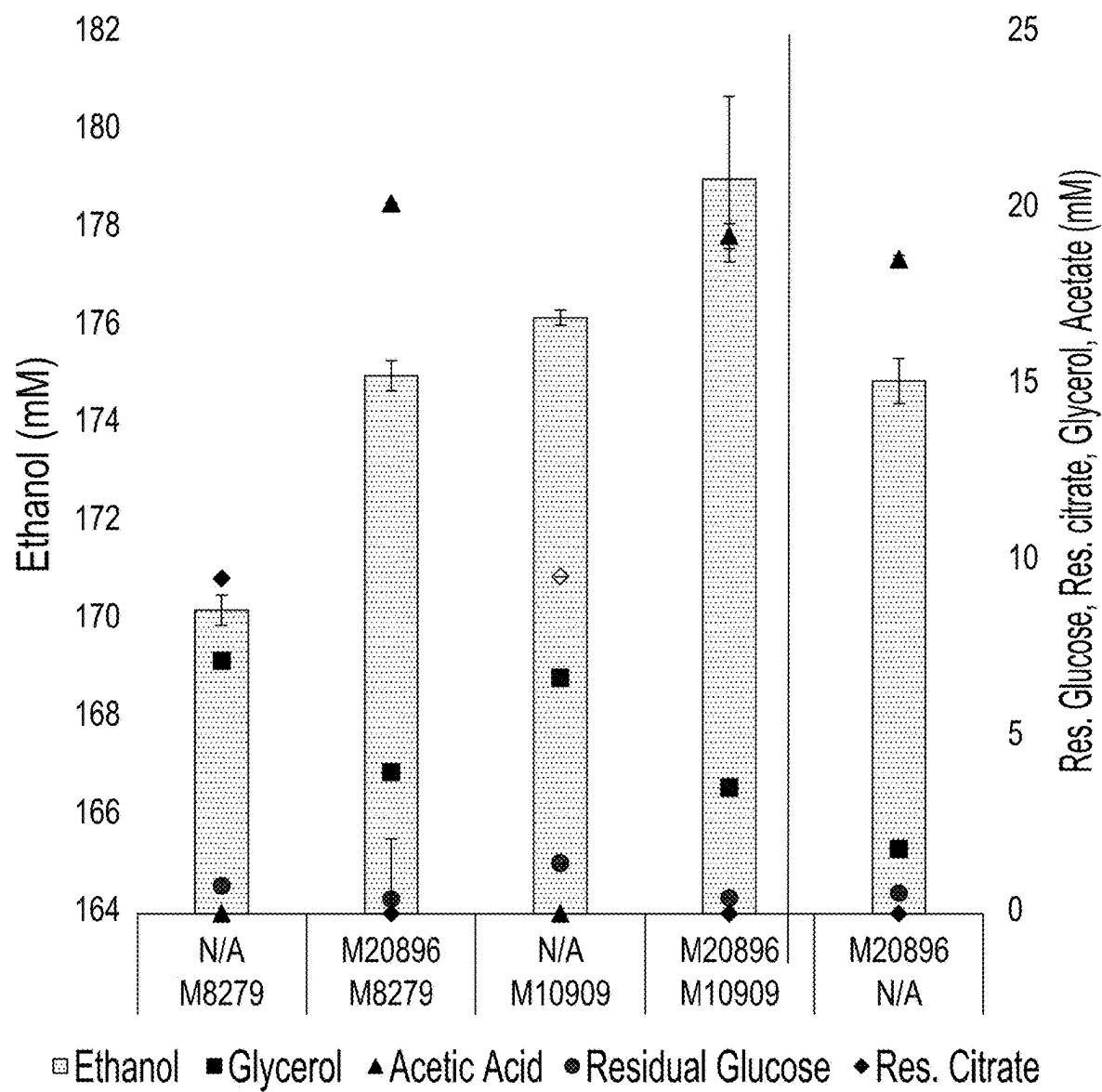


Figure 10

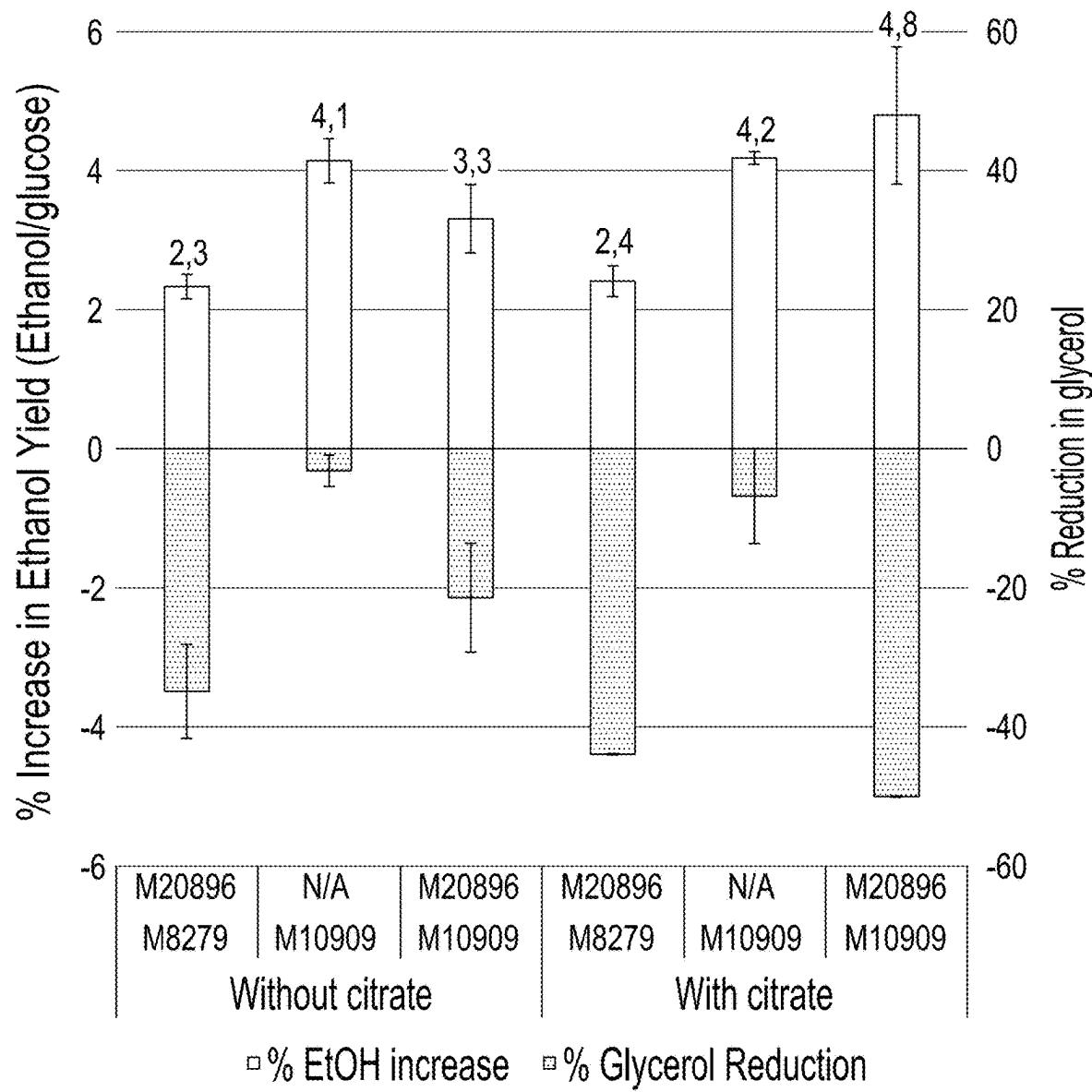


Figure 11

SYNERGISTIC BACTERIAL AND YEAST COMBINATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation of U.S. patent application Ser. No. 17/292,358, filed on May 7, 2021, which is a U.S. national phase application of PCT/IB2019/059765 filed on Nov. 13, 2019, which claims priority from U.S. provisional patent application 62/760,472 filed on Nov. 13, 2018 all of which are herewith incorporated by reference in their entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence (580127_423C1_SEQUENCE_LISTING.xml.; Size 101,601 bytes; and Date of Creation: Dec. 3, 2024) is herein incorporated by reference in its entirety.

TECHNOLOGICAL FIELD

[0003] The present disclosure concerns a combination of a bacterial host cell and a yeast host cell exhibiting a symbiotic relationship to convert a first metabolic product into a second metabolic product.

BACKGROUND

[0004] Interactions between various microorganisms have been well characterized in numerous diverse environments, ranging from food and beverage production to clinical settings. These interactions can be either antagonistic or symbiotic in nature and play a significant role in the balance of microbial ecosystems. Symbiotic interactions may be mutualistic, wherein both organisms benefit, or commensal, where only one benefits. One example of a symbiotic relationship includes the production and secretion of metabolites by one organism that are utilized by another (Schink, 2002). The subsequent organism benefits either due to their lack of the enzymes required for the synthesis of the metabolite or through conservation of energy that would otherwise be required to synthesize it de novo.

[0005] These microbial interactions occur both within and across phylogenetic kingdoms and several reports of yeast-bacterial interactions have been documented (Peleg et al., 2010; Wargo and Hogan, 2006). The yeast, *Saccharomyces cerevisiae*, is utilized as the primary bio-catalyst in commercial bioethanol production, however, diverse populations of lactic acid bacteria (LAB) are also ubiquitous within the fermentation vessels. The impacts of LAB on yeast fermentation have typically been shown to be antagonistic leading to decreased ethanol titers and stuck fermentations. Antibiotics are therefore heavily utilized within the industry to try and mitigate infections. However, the use of antibiotics raises concerns related to the selection of resistant bacterial strains and the presence of antibiotics in fermentation residuals that are sold as animal feed.

[0006] For instance, *Lactobacillus paracasei* strain 12A robustly utilizes trehalose even when glucose is readily available. Trehalose is a common constituent of residual DP2 sugars (sugars with degree of polymerization=2) in corn fermentations. *Saccharomyces cerevisiae* often synthesizes trehalose in response to stress and previous studies have indicated that up-regulation of trehalose biosynthesis

improves yeast robustness. Unfortunately, trehalose accumulation by the yeast is known to subtract from ethanol yield as glucose-6-phosphate is diverted from central metabolism through the enzymes TPS1 and TPS2 (Yi et al., 2016).

[0007] It would be highly desirable to be provided with means of increasing alcohol production during yeast fermentation that would exploit, rather than limit, the symbiotic relationship between yeasts and bacteria, especially lactic acid bacteria.

BRIEF SUMMARY

[0008] The present disclosure concerns a symbiotic combination of a yeast host cell and a bacterial host cell. The symbiotic combination cell has the ability or is engineered to make a first metabolic product intended to be used by the second microbial host cell to make a second metabolic product. In some embodiments, the symbiotic combination achieve higher fermentation yield (when compared for example from a fermentation conducted in the absence of the bacterial cell). In some embodiments, the symbiotic combination of the present disclosure provides higher robustness.

[0009] According to a first aspect, the present disclosure provides a combination of a first microbial host cell having a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product and a second microbial host cell having a second metabolic pathway comprising one or more second enzymes for converting at least in part the first metabolic product into a second metabolic product. In such combination, at least one of the first microbial host cell or the second microbial host cell is recombinant; at least one of the first microbial host cell or the second microbial host cell is a bacterial host cell; and at least one of the first microbial host cell or the second microbial host cell is a yeast host cell. In the combinations of the present disclosure, when the first microbial host cell is a recombinant first microbial host cell, the recombinant first microbial host cell has increased activity in the first metabolic pathway, when compared to a corresponding native first microbial host cell, for producing the first metabolic product. Still in the combinations of the present disclosure, when the second microbial host cell is a recombinant second microbial host cell, the recombinant second microbial host cell has increased activity in the second metabolic pathway, when compared to a corresponding native second microbial host cell, for converting at least in part the first metabolic product into the second metabolic product. In an embodiment, the first microbial host cell is a bacterial host cell and the second microbial cell is a yeast host cell. As such, the present disclosure provides a combination of a bacterial host cell having a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product and a yeast host cell having a second metabolic pathway comprising one or more second enzymes for converting at least in part the first metabolic product into a second metabolic product, wherein at least one of the bacterial host cell or the yeast host cell is recombinant. When the bacterial host cell is a recombinant bacterial host cell, the recombinant bacterial host cell has increased activity in the first metabolic pathway, when compared to a corresponding native bacterial host cell, for producing the first metabolic product. When the yeast host cell is a recombinant yeast host cell, the recombinant yeast

host cell has increased activity in the second metabolic pathway, when compared to a corresponding native yeast host cell, for converting at least in part the first metabolic product into the second metabolic product. In an embodiment, at least one of the one or more first enzymes are native enzymes. In another embodiment, at least one of the one or more second enzymes are heterologous enzymes. In an embodiment, the first metabolic product is an organic ester, such as, for example, acetate. In another embodiment, the second metabolic product is ethanol. In an embodiment, the one or more first enzymes comprises a citrate lyase.

[0010] In some embodiments, the yeast host cell is the recombinant yeast host cell and the one or more second enzyme comprises a polypeptide having an heterologous polypeptide having acetylating acetaldehyde dehydrogenase activity. The polypeptide having acetylating acetaldehyde dehydrogenase activity is an acetylating acetaldehyde dehydrogenase (AADH) or a bifunctional acetylating ace/alcohol dehydrogenase (ADHE). In specific embodiments, the polypeptide having acetylating aldehyde dehydrogenase activity is heterologous bifunctional acetaldehyde/alcohol dehydrogenase (ADHE) having, in some embodiments, the amino acid sequence of SEQ ID NO: 15, being a variant of the amino acid sequence of SEQ ID NO: 15 having acetaldehyde/alcohol dehydrogenase activity or being a fragment of the amino acid sequence of SEQ ID NO: 15 having acetaldehyde/alcohol dehydrogenase activity. In some embodiments, the one or more second enzymes comprises an heterologous polypeptide having NADP⁺-dependent alcohol dehydrogenase activity (e.g., NADPH-ADH which can be, for example, ADH1 which can be obtained from *Entamoeba* sp., including *Entamoeba nuttalli*) or a polypeptide encoded by an adhl gene ortholog). In an embodiment, heterologous polypeptide having NADP⁺-dependent alcohol dehydrogenase activity has the amino acid sequence of SEQ ID NO: 45, is a variant of the amino acid sequence of SEQ ID NO: 45 exhibiting NADP⁺-dependent alcohol dehydrogenase activity or is a fragment of the amino acid sequence of SEQ ID NO: 45 exhibiting NADP⁺-dependent alcohol dehydrogenase activity. In some embodiments, the one or more second enzymes comprise an heterologous polypeptide having acetyl-coA synthetase activity (which can be, for example ACS2 or a polypeptide encoded by an acs2 gene ortholog). In an embodiment, the heterologous polypeptide having acetyl-coA synthetase activity has the amino acid sequence of SEQ ID NO: 49, is a variant of the amino acid sequence of SEQ ID NO: 49 exhibiting acetyl-coA synthetase activity or is a fragment of the amino acid sequence of SEQ ID NO: 49 exhibiting acetyl-coA synthetase activity.

[0011] In some embodiments, the first microbial host cell is a yeast host cell and the second microbial host cell is a bacterial host cell. As such, the present disclosure provides a combination of a yeast host cell having a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product and a bacterial host cell having a second metabolic pathway comprising one or more second enzymes for converting at least in part the first metabolic product into a second metabolic product, wherein at least one of the yeast host cell or the bacterial host cell is recombinant. When the yeast host cell is a recombinant yeast host cell, the recombinant yeast host cell has increased activity in the first metabolic pathway, when compared to a corresponding native yeast host cell, for producing the first metabolic product. When the bacterial host cell is a recom-

binant bacterial host cell, the recombinant bacterial host cell has increased activity in the second metabolic pathway, when compared to a corresponding native bacterial host cell, for converting at least in part the first metabolic product into the second metabolic product. In an embodiment, at least one of the one or more first enzymes are heterologous enzymes. In another embodiment, at least one of the one or more second enzymes are heterologous enzymes. In an embodiment, the first metabolic product is a carbohydrate. In another embodiment, the second metabolic product is ethanol.

[0012] In a specific embodiment, the carbohydrate is trehalose. In such embodiment, the one or more first enzymes comprises a trehalose-6-phosphate synthase, such as, for example, TPS1. In such embodiment, the one or more first enzymes comprises a trehalose-6-phosphate phosphatase, such as, for example, TPS2. In such embodiment, the one or more second enzymes comprises a pyruvate decarboxylase. The pyruvate decarboxylase can have, in some embodiments, the amino acid sequence of SEQ ID NO: 4, be a variant of the amino acid sequence of SEQ ID NO: 4 having pyruvate decarboxylase activity or be a fragment of the amino acid sequence of SEQ ID NO: 4 having pyruvate decarboxylase activity. In such embodiments, the one or more second enzymes comprises an alcohol dehydrogenase. The alcohol dehydrogenase can have, in some embodiments, the amino acid sequence of SEQ ID NO: 8, be a variant of the amino acid sequence of SEQ ID NO: 8 having alcohol dehydrogenase activity or be a fragment of the amino acid sequence of SEQ ID NO: 8 having alcohol dehydrogenase activity. In an embodiment, the bacterial host cell has a decreased lactate dehydrogenase activity when compared to the corresponding native bacterial host cell. In a further embodiment, the bacterial host cell has at least one inactivated native gene coding for a lactate dehydrogenase, such as, for example ldh1, ldh2, ldh3 or ldh4. In yet another embodiment, the bacterial host cell has a decreased mannitol dehydrogenase activity. In some embodiments, the bacterial host cell has at least one inactivated native gene coding for a mannitol-1-phosphate 5-dehydrogenase, such as, for example, mltD1 or mltD2.

[0013] In another specific embodiment, the carbohydrate is mannitol. In such embodiment, the one or more first enzymes comprises a mannitol-1-phosphate 5-dehydrogenase. In such embodiment, the one or more first enzymes comprises a MTLD enzyme. In some embodiments, the MTLD polypeptide can have the amino acid sequence of SEQ ID NO: 27, be a variant of the amino acid sequence of SEQ ID NO: 27 or be a fragment of the amino acid sequence of SEQ ID NO: 27 or a variant thereof. In some additional embodiments, the MTLD polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 28, a variant of the nucleic acid sequence of SEQ ID NO: 28 or a fragment of the nucleic acid sequence of SEQ ID NO: 28 or a fragment thereof. In such embodiment, the one or more second enzymes comprise at least one gene from a mannitol utilization operon. In yet another embodiment, the one or more second enzymes comprise mannitol-1-phosphatase 5-dehydrogenase. In still another embodiment, the one or more second enzymes comprise a MTLD2 polypeptide. In an embodiment, the MTLD2 polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLD2 polypeptide can have the amino acid sequence of

SEQ ID NO: 39, be a variant of the amino acid sequence of SEQ ID NO: 39 or be a fragment of the amino acid sequence of SEQ ID NO: 39 or a variant thereof. In some additional embodiments, the MTLD2 polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 40, a variant of the nucleic acid sequence of SEQ ID NO: 40 or a fragment of the nucleic acid sequence of SEQ ID NO: 40 or a fragment thereof.

[0014] In another embodiment, the one or more second enzymes comprises a mannitol transporter. In some embodiments, the mannitol transporter comprises at least one of the MTLCB polypeptide or the MTLA polypeptide. In an embodiment, the MTLCB polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLCB polypeptide can have the amino acid sequence of SEQ ID NO: 41, be a variant of the amino acid sequence of SEQ ID NO: 41 or be a fragment of the amino acid sequence of SEQ ID NO: 41 or a variant thereof. In some additional embodiments, the MTLCB polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 42, a variant of the nucleic acid sequence of SEQ ID NO: 42 or a fragment of the nucleic acid sequence of SEQ ID NO: 42 or a fragment thereof. In an embodiment, the MTLA polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLA polypeptide can have the amino acid sequence of SEQ ID NO: 43, be a variant of the amino acid sequence of SEQ ID NO: 43 or be a fragment of the amino acid sequence of SEQ ID NO: 43 or a variant thereof. In some additional embodiments, the MTLA polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 44, a variant of the nucleic acid sequence of SEQ ID NO: 44 or a fragment of the nucleic acid sequence of SEQ ID NO: 44 or a fragment thereof.

[0015] In another specific embodiment, the carbohydrate is sorbitol. In such embodiment, the one or more first enzymes comprises a sorbitol-6-phosphate dehydrogenase (SRLD). In an embodiment, the one or more first enzymes comprises a SRLD enzyme. In still another embodiment, the one or more second enzymes comprises at least one gene from a sorbitol utilization operon, such as, for example, at least one of a gutF, a gutC, a gutB and/or a gutA gene. In an embodiment, the GUTF polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTF polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 31, be a variant of the amino acid sequence of SEQ ID NO: 31 or be a fragment of the amino acid sequence of SEQ ID NO: 31 or a variant thereof. In an embodiment, the GUTF polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 32, being a variant of the nucleic acid sequence of SEQ ID NO: 32 or being a fragment of the nucleic acid sequence of SEQ ID NO: 32 or a variant thereof. In an embodiment, the GUTC polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTC polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 33, be a variant of the amino acid sequence of SEQ ID NO: 33 or be a fragment of the amino acid sequence of SEQ ID NO: 33 or a variant thereof. In an embodiment, the GUTC polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO:

34, being a variant of the nucleic acid sequence of SEQ ID NO: 34 or being a fragment of the nucleic acid sequence or SEQ ID NO: 34 or a variant thereof. In an embodiment, the GUTB polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTB polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 35, be a variant of the amino acid sequence of SEQ ID NO: 35 or be a fragment of the amino acid sequence of SEQ ID NO: 35 or a variant thereof. In an embodiment, the GUTB polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 36, being a variant of the nucleic acid sequence of SEQ ID NO: 36 or being a fragment of the nucleic acid sequence or SEQ ID NO: 36 or a variant thereof. In an embodiment, the GUTA polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTA polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 37, be a variant of the amino acid sequence of SEQ ID NO: 37 or be a fragment of the amino acid sequence of SEQ ID NO: 37 or a variant thereof. In an embodiment, the GUTA polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 38, being a variant of the nucleic acid sequence of SEQ ID NO: 38 or being a fragment of the nucleic acid sequence or SEQ ID NO: 38 or a variant thereof.

[0016] In another specific embodiment, the carbohydrate is glycerol. In an embodiment, the second metabolic pathway comprises a glycerol dehydrogenase/DHA kinase pathway. In such embodiment, the one or more second enzymes comprise at least one of a glycerol dehydrogenase or a dihydroxyacetone kinase. In another embodiment, the second metabolic pathway comprises a glycerol kinase/glycerol-3-phosphate dehydrogenase pathway. In such embodiment, the one or more second enzymes comprise at least one of a glycerol kinase or a glycerol-3-phosphate dehydrogenase. In such embodiment, the one or more second enzymes comprises a glycerol facilitator. In an embodiment, the yeast host cell has increased activity, when compared to the corresponding native yeast host cell, in an NADP⁺-dependent aldehyde dehydrogenase, such as, for example ALD6. In embodiment, the yeast host cell has increased activity, when compared to the corresponding native yeast host cell, in a phosphoketolase.

[0017] In the combinations of the present disclosure, the yeast host cell can be from *Saccharomyces* sp., such as, for example, *Saccharomyces cerevisiae*. In an embodiment, the bacterial host cell is a lactic acid bacterium.

[0018] In some embodiments, the bacterial host cell further comprises a third metabolic pathway comprising one or more third enzymes for producing a third metabolic product. In such embodiment, the bacterial host cell is the recombinant bacterial host cell and has increased activity in the third metabolic pathway, when compared to the corresponding native bacterial host cell, for producing the third metabolic product. In some embodiments, the third metabolic product is ethanol. In some additional embodiments, the one or more third enzymes for producing the third metabolic product comprises a pyruvate decarboxylase. In some embodiments, the pyruvate decarboxylase has the amino acid sequence of SEQ ID NO: 4, is a variant of the amino acid sequence of SEQ ID NO: 4 having pyruvate decarboxylase activity or is a fragment of the amino acid sequence of SEQ ID NO: 4 having pyruvate decarboxylase activity. In yet another

embodiment, the one or more third enzymes comprises an alcohol dehydrogenase. In some embodiments, the alcohol dehydrogenase has the amino acid sequence of SEQ ID NO: 8, is a variant of the amino acid sequence of SEQ ID NO: 8 having alcohol dehydrogenase activity or is a fragment of the amino acid sequence of SEQ ID NO: 8 having alcohol dehydrogenase activity. In yet another embodiment, the bacterial host cell has a decreased lactate dehydrogenase activity when compared to the corresponding native bacterial host cell. In specific embodiments, the bacterial host cell has at least one inactivated native gene coding for a lactate dehydrogenase, such as, for example, ldh1, ldh2, ldh3 or ldh4. In some embodiments, the bacterial host cell has decreased mannitol dehydrogenase activity. In specific embodiments, the bacterial host cell has at least one inactivated native gene coding for a mannitol-1-phosphate 5-dehydrogenase, such as, for example, mltD1 or mltD2.

[0019] The bacterial host cell can be from *Lactobacillus* sp., such as, for example, *Lactobacillus paracasei*. The yeast host cell and/or the bacterial host cell can be provided as a cell concentrate. For example, the yeast host cell can be provided as a cream. In another example, the bacterial host cell can be provided as a frozen cell concentrate.

[0020] According to a third aspect, the present disclosure provides a process for converting a biomass into a fermentation product, the process comprises contacting the biomass with the combination defined herein under condition to allow the conversion of at least a part of the biomass into the fermentation product. In an embodiment, the biomass comprises corn, such as, for example, a corn provided as a mash. In another embodiment, the biomass comprises or is supplemented with citric acid and/or citrate. In an embodiment, the fermentation product is ethanol. In yet another embodiment, the process is conducted, at least in part, at a temperature higher than 31° C.

[0021] According to a fourth aspect, the present disclosure provides a commercial package comprising (i) the combination defined herein and (ii) instructions to perform the process defined herein. In an embodiment, the commercial package further comprises a fermentation medium comprising a biomass, such as, for example, a biomass comprising corn. In another embodiment, the commercial package further comprises citric acid and/or citrate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Having thus generally described the nature of the invention, reference will now be made to the accompanying drawings, showing by way of illustration, a preferred embodiment thereof, and in which:

[0023] FIG. 1 illustrates an embodiment of a metabolic engineering strategy for trehalose production by yeast host cell and subsequent metabolism by a bacterial host cell. Pathway components in black solid lines represent metabolic reactions that occur in the yeast host cell and the bacterial host cell to produce ethanol from glucose. The pathway identified by dotted lines (from glucose-6-P to trehalose) is used to promote trehalose production by the yeast host cell, and the pathway identified by dashed lines (from trehalose to glucose-6-P) shows how trehalose is metabolized by the bacterial host cell.

[0024] FIG. 2 illustrates an embodiment of a metabolic engineering strategy for utilization of yeast-derived glycerol by a bacterial host cell. The pathway identified black solid lines font represent metabolic reactions that occur in the

yeast host cell and the bacterial host cell to produce ethanol from glucose. The pathway identified by dotted lines (from dihydroxyacetone-P to glycerol) is used by yeast host cell for glycerol production, and the pathway identified in dashed lines (from glycerol to dihydroxyacetone-P) shows strategies used to metabolically engineer the bacterial host cell to metabolize glycerol.

[0025] FIG. 3 illustrates an embodiment of a metabolic engineering strategy for mannitol production by a yeast host cell and subsequent metabolism by a bacterial host cell. The pathway components in solid font represent metabolic reactions that occur in the yeast and the bacterial host cell to produce ethanol from glucose. The pathway identified in dotted lines (from fructose-6-P to mannitol) is used to promote mannitol production by the yeast host cell, and the pathway identified by the dashed lines (from mannitol to fructose-6-P) shows how mannitol can be metabolized by the bacterial host cell.

[0026] FIG. 4 illustrates an embodiment of a metabolic engineering strategy for sorbitol production by yeast host cell and subsequent metabolism by a bacterial host cell. The pathway components in black solid font represent metabolic reactions that occur in the yeast and the bacterial host cells to produce ethanol from glucose. The pathway identified in dotted lines (from fructose to sorbitol) is used to promote sorbitol production by the yeast host cell, and the pathway identified by dashed lines (from sorbitol to fructose) shows how sorbitol is metabolized by the bacterial host cell.

[0027] FIG. 5 illustrates that improved yeast robustness can be achieved from both trehalose overexpression and co-fermentation with ethanologen strain E3.1. Ethanol (left Y axis in g/L, bars) and glucose (right Y axis in g/L, ♦) concentrations following 50 hours of fermentation in commercial corn mash are shown in both standard (permissive) and high temperature conditions. Strain M12156 was not modified to produce additional amounts of trehalose, while strain M16807 was modified to produce additional amounts of trehalose (by expressing TPS1 and TPS2) (refer to Table 1 for a description of the strains used).

[0028] FIG. 6 illustrates improved fermentation yield can be achieved from both sorbitol overexpression and co-fermentation with ethanologen strain M19605. Ethanol (left Y axis in g/L, bars), glucose (right Y axis in mM, ●), glycerol (right axis in mM, ■) and sorbitol (right axis in mM, ▲) concentrations following 67 hours of fermentation in a modified chemically defined medium are shown. Results are shown with respect to the strains or combination of strains tested. Strain M2390 is a wild-type strain, while strain M20043 has been modified to express SRLD (see Table 4 for a description of the strains used).

[0029] FIG. 7 illustrates improved fermentation yield can be achieved from both mannitol overexpression and co-fermentation with ethanologen strain M19998. Ethanol (left Y axis in g/L, bars), glucose (right Y axis in mM, ●), glycerol (right axis in mM, ■) and mannitol (right axis in mM, ▲) concentrations following 67 hours of fermentation in a modified chemically defined medium are shown. Results are shown with respect to the strains or combination of strains tested. Strain M2390 is a wild-type strain, while strain M20036 has been modified to express MTLD (see Table 4 for a description of the strains used).

[0030] FIG. 8 illustrates an embodiment of a metabolic engineering strategy for utilization of bacterial-derived citrate by a yeast host cell. The pathway identified black solid

lines font represent metabolic reactions that occur in the yeast and the bacterial host cell. The pathway identified by dotted lines (from acetate to acetaldehyde) is used by yeast host cell for ethanol production, and the pathway identified in dashed lines (from citrate to acetate) shows strategies used to metabolically engineer the bacterial host cell to metabolize citrate.

[0031] FIG. 9 illustrates the metabolite profiles of *Lb. paracasei* 12A and derived ethanologen E5 in after fermentation for 68 h in mCDM medium supplemented with 50 mM glucose (pH 6.5). Results are shown as the net mM of glucose, lactic acid, acetic acid, ethanol and citric acid in function of the strain tested.

[0032] FIG. 10 illustrates the metabolite profiles of *S. cerevisiae* strains M8279 and M10909 (alone or in combination with *Lb. paracasei* strain M20896) after fermentation for 68 h in mCDM medium supplemented with 50 mM glucose (pH 6.5). Results are shown as the net mM of ethanol (left axis), glycerol acetic acid, residual glucose and residual citrate in function of the strain tested.

[0033] FIG. 11 illustrates the percent increase in ethanol yield (ethanol/glucose) and percent glycerol reduction of *S. cerevisiae* strains M8279 and M10909 (alone or in combination with *Lb. paracasei* strain M20896) after fermentation for 68 h in mCDM medium supplemented with 50 mM glucose (pH 6.5) without and with the presence of citrate. Results are shown as the percent increase in ethanol yield (ethanol/glucose, left axis) and percent glycerol reduction in function of the strain tested and the presence or absence of citrate.

DETAILED DESCRIPTION

[0034] The present disclosure concerns a combination of a yeast host cell and a bacterial host cell wherein one of the host cell is a recombinant host cell. One of the host cell has a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product. The other host cell has a second metabolic pathway comprising one or more second enzymes for converting (at least in part) the first metabolic product into a second metabolic product. In an embodiment, the combination provides increased robustness to the yeast host cell in response to a stressor, such as for example elevated temperatures.

[0035] In some embodiments of the combinations of the present disclosure, the yeast host cell has the ability or is engineered to make a first metabolic product intended to be utilized by the bacterial host cell (to make the second metabolic product). When the yeast host cell is recombinant (e.g., engineered to make the first metabolic product), it has an increased activity in the first metabolic pathway when compared to the native or parental yeast host cell (which has been used to engineer the recombinant yeast host cell and which lacks the genetic modification(s) associated to increase the activity in the first metabolic pathway). In such embodiment, the bacterial host cell has the ability or is engineered to make a second metabolic product from the first metabolic product produced at least in part by the yeast host cell. When the bacterial host cell is recombinant (e.g., engineered to make the second metabolic product), it has an increased activity in the second metabolic pathway when compared to the native or parental bacterial host cell (which has been used to engineer the recombinant bacterial host cell and which lacks the genetic modification(s) associated to increase the activity in the second metabolic pathway). In

specific embodiments, the first metabolic product is made from a molecule that is used to produce a fermentation product (an alcohol such as ethanol).

[0036] In alternative embodiments of the combinations of the present disclosure, the bacterial host cell has the ability or is engineered to make a first metabolic product intended to be utilized by the yeast host cell (to make the second metabolic product). When the bacterial host cell is recombinant (e.g., engineered to make the first metabolic product), it has an increased activity in the first metabolic pathway when compared to the native or parental bacterial host cell (which has been used to engineer the recombinant bacterial host cell and which lacks the genetic modification(s) associated to increase the activity in the first metabolic pathway). In such embodiment, the yeast host cell has the ability or is engineered to make a second metabolic product from the first metabolic product produced at least in part by the bacterial host cell. When the yeast host cell is recombinant (e.g., engineered to make the second metabolic product), it has an increased activity in the second metabolic pathway when compared to the native or parental yeast host cell (which has been used to engineer the recombinant yeast host cell and which lacks the genetic modification(s) associated to increase the activity in the second metabolic pathway). In specific embodiments, the first metabolic product is made from a molecule that is used to produce a fermentation product (an alcohol such as ethanol).

[0037] In specific embodiments, the second metabolic product can be used in the production of a fermentation product (an alcohol such as ethanol). In some embodiments, the combinations of the present disclosure are useful for recycling a yeast osmo-protectant (trehalose, mannitol, sorbitol and/or glycerol for example) into a fermentation product (such as ethanol). In some embodiments, the yeast/bacterial relationship promotes the production of a fermentation product, such as, for example, an alcohol (e.g., ethanol).

[0038] In one embodiment, shown on FIG. 1, the first metabolic product produced by the yeast host cell can be trehalose which can subsequently be metabolized to ethanol (e.g., the second metabolic product) by the bacterial host cell. When the second metabolic product is ethanol, the yeast host cell can be selected based on its ability to convert glucose-6-phosphate into α,α -trehalose-6-phosphate (α,α -trehalose-6-P), α,α -trehalose-6-P into trehalose (via the activity of one or more a trehalose-6-phosphatase). In some embodiments, the yeast host cell can be genetically modified to provide or increase its ability to convert glucose-6-phosphate into α,α -trehalose-6-phosphate (α,α -trehalose-6-P) and/or α,α -trehalose-6-P into trehalose (via the activity of one or more a trehalose-6-phosphatase). In the embodiment shown on FIG. 1, when the second metabolic product is ethanol, the bacterial host cell can be selected based on its ability to convert trehalose into trehalose-6-phosphate (trehalose-6-P, via the activity or one or more PTS transporter), trehalose-6-P into glucose and glucose-6-P (via the activity of one or more trehalose-6-phosphate hydrolase) and glucose into glucose-6-P (via the activity of one or more hexokinase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to convert trehalose into trehalose-6-phosphate (trehalose-6-P, via the activity or one or more PTS transporter), trehalose-6-P into glucose and glucose-6-P (via the activity of one or more trehalose-6-phosphate hydrolase) and/or glucose into

glucose-6-P (via the activity of one or more hexokinase). In another embodiment shown on FIG. 1, when the second metabolic product is ethanol, the bacterial host cell can be selected based on its ability to convert pyruvate into acet-aldehyde (via the activity or one or more pyruvate decarboxylase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to convert pyruvate into acetaldehyde (via the activity or one or more pyruvate decarboxylase). In yet another embodiment shown on FIG. 1, when the second metabolic product is ethanol, the bacterial host cell can be selected based on its ability to convert acetaldehyde into ethanol (via the activity or one or more alcohol dehydrogenase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to convert acetaldehyde into ethanol (via the activity or one or more alcohol dehydrogenase).

[0039] In another embodiment, shown on FIG. 2, the first metabolic product produced by the yeast host cell can be glycerol which can subsequently be metabolized to ethanol production (e.g., the second metabolic product) by the bacterial host cell. In such embodiment, the yeast host cell can be selected based on its ability to convert dihydroxyacetone-P into glycerol-3-phosphate (glycerol-3-P, via the activity of one or more dihydroxyacetone-3-P dehydrogenase), glycerol-3-P into glycerol (via the activity of one or more a glycerol-3-P phosphatase). In some embodiments, the yeast host cell can be genetically modified to provide or increase its ability to convert dihydroxyacetone-P into glycerol-3-phosphate (glycerol-3-P, via the activity of one or more dihydroxyacetone-3-P dehydrogenase) and/or glycerol-3-P into glycerol (via the activity of one or more a glycerol-3-P phosphatase). In the embodiment shown on FIG. 2, the bacterial host cell can be selected based on its ability to import glycerol (via the activity or one or more glycerol facilitator), to convert glycerol into glycerol-3-P (via the activity of one or more glycerol kinase), glycerol-3-P into dihydroxyacetone-P (via the activity of one or more glycerol-3-P dehydrogenase), glycerol into dihydroxyacetone (via the activity of one or more glycerol dehydrogenase) and dihydroxyacetone into dihydroxyacetone-P (via the activity or one or more dihydroxyacetone kinase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to import glycerol (via the activity or one or more glycerol facilitator), to convert glycerol into glycerol-3-P (via the activity of one or more glycerol kinase), glycerol-3-P into dihydroxyacetone-P (via the activity of one or more glycerol-3-P dehydrogenase), glycerol into dihydroxyacetone (via the activity of one or more glycerol dehydrogenase) and/or dihydroxyacetone into dihydroxyacetone-P (via the activity or one or more dihydroxyacetone kinase).

[0040] In another embodiment, shown on FIG. 3, the first metabolic product produced by the yeast host cell can be mannitol which can subsequently be metabolized to ethanol (e.g., the second metabolic product) by the bacterial host cell. In such embodiment, the yeast host cell can be selected based on its ability to convert fructose-6-P into mannitol-1-phosphate (mannitol-1-P, via the activity of one or more mannitol dehydrogenase) and mannitol-1-P into mannitol (via the activity of one or more a mannitol-1-P phosphatase). In some embodiments, the yeast host cell can be genetically modified to provide or increase its ability to convert fructose-6-P into mannitol-1-phosphate (mannitol-1-P, via the

activity of one or more mannitol dehydrogenase) and/or mannitol-1-P into mannitol (via the activity of one or more a mannitol-1-P phosphatase). In the embodiment shown on FIG. 3, the bacterial host cell can be selected based on its ability to convert mannitol into mannitol-1-phosphate (mannitol-1-P, via the activity of one or more PTS transporter) and mannitol-1-P into fructose-6-P (via the activity of one or more mannitol dehydrogenase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to convert mannitol into mannitol-1-phosphate (mannitol-1-P, via the activity of one or more PTS transporter) and/or mannitol-1-P into fructose-6-P (via the activity of one or more mannitol dehydrogenase).

[0041] In another embodiment, shown on FIG. 4, the first metabolic product produced by the yeast host cell can be sorbitol which can subsequently be metabolized to ethanol (e.g., the second metabolic product) by the bacterial host cell. In such embodiment, the yeast host cell can be selected based on its ability to convert fructose-6-P into sorbitol-6-phosphate (sorbitol-6-P, via the activity of one or more sorbitol dehydrogenase) and sorbitol-6-P into sorbitol (via the activity of one or more a sorbitol-6-P phosphatase). In some embodiments, the yeast host cell can be genetically modified to provide or increase its ability to convert fructose-6-P into sorbitol-6-phosphate (sorbitol-6-P, via the activity of one or more sorbitol dehydrogenase) and/or sorbitol-6-P into sorbitol (via the activity of one or more a sorbitol-6-P phosphatase). In the embodiment shown on FIG. 4, the bacterial host cell can be selected based on its ability to convert sorbitol into sorbitol-6-phosphate (sorbitol-6-P, via the activity of one or more PTS transporter) and sorbitol-6-P into fructose-6-P (via the activity of one or more sorbitol dehydrogenase). In some embodiments, the bacterial host cell is genetically modified to provide or increase its ability to convert sorbitol into sorbitol-6-phosphate (sorbitol-6-P, via the activity of one or more PTS transporter) and/or sorbitol-6-P into fructose-6-P (via the activity of one or more sorbitol dehydrogenase).

[0042] In a further embodiment, shown on FIG. 8, the first metabolic product produced by the bacterial host cell can be acetic acid (or acetate) which can subsequently be metabolized to ethanol (e.g., the second metabolic product) by the yeast host cell. In the embodiment shown on FIG. 8, the bacterial host cell is capable of producing acetate which can further be hydrolyzed into acetic acid in subsequent steps. Still in the embodiment shown on FIG. 8, the bacterial host cell can be selected based on its ability to convert citric acid (or its associated ester citrate) into acetic acid (or its associated ester acetate) (via the activity of one or more citrate lyase). In some embodiments, the bacterial host cell can be genetically modified to provide or increase its ability to convert citric acid (citrate) into acetic acid (acetate) (via the activity of one or more citrate lyase). In the embodiment shown on FIG. 8, the yeast host cell can be selected based on its ability to convert acetic acid (acetate) into acetyl-CoA, via the activity of one or more acetyl-CoA synthetase (such as for example ACS2). In some embodiments, the yeast host cell is genetically modified to provide or increase its ability to convert acetic acid (acetate) into acetyl-CoA, via the activity of one or more acetyl-CoA synthetase (such as for example ACS2). Still in the embodiment shown on FIG. 8, the yeast host cell can be selected based on its ability to convert acetyl-CoA into acetaldehyde, via the activity of one or more bifunctional acetylating aldehyde dehydrogenase/

alcohol dehydrogenase (such as for example ADHE). In some embodiments, the yeast host cell is genetically modified to provide or increase its ability to convert acetyl-coA into acetaldehyde, via the activity of one or more bifunctional acetylating aldehyde dehydrogenase/alcohol dehydrogenase (such as for example ADHE).

[0043] The combination of the present disclosure comprises a recombinant yeast host cell and/or a recombinant bacterial host cells. These recombinant host cells can be obtained by introducing one or more genetic modifications in a corresponding native (parental) yeast/bacterial host cell. When the genetic modification is aimed at reducing or inhibiting the expression of a specific targeted gene (which is endogenous to the host cell), the genetic modifications can be made in one or both copies of the targeted gene(s). When the genetic modification is aimed at increasing the expression of a specific targeted gene, the genetic modification can be made in one or multiple genetic locations. In the context of the present disclosure, when recombinant yeast and bacterial host cells are qualified as being “genetically engineered”, it is understood to mean that they have been manipulated to either add at least one or more heterologous or exogenous nucleic acid residue and/or removed at least one endogenous (or native) nucleic acid residue. In some embodiments, the one or more nucleic acid residues that are added can be derived from an heterologous cell or the recombinant host cell itself. In the latter scenario, the nucleic acid residue(s) is (are) added at a genomic location which is different than the native genomic location. The genetic manipulations did not occur in nature and are the results of *in vitro* manipulations of the native yeast or bacterial host cell.

[0044] When expressed in recombinant host cells, the polypeptides (including the enzymes) described herein are encoded on one or more heterologous nucleic acid molecule. The term “heterologous” when used in reference to a nucleic acid molecule (such as a promoter or a coding sequence) refers to a nucleic acid molecule that is not natively found in the recombinant host cell. “Heterologous” also includes a native coding region, or portion thereof, that is removed from the source organism and subsequently reintroduced into the source organism in a form that is different from the corresponding native gene, e.g., not in its natural location in the organism’s genome. The heterologous nucleic acid molecule is purposively introduced into the recombinant host cell. The term “heterologous” as used herein also refers to an element (nucleic acid or protein) that is derived from a source other than the endogenous source. Thus, for example, a heterologous element could be derived from a different strain of host cell, or from an organism of a different taxonomic group (e.g., different kingdom, phylum, class, order, family genus, or species, or any subgroup within one of these classifications). The term “heterologous” is also used synonymously herein with the term “exogenous”.

[0045] When an heterologous nucleic acid molecule is present in the recombinant host cell, it can be integrated in the host cell’s genome. The term “integrated” as used herein refers to genetic elements that are placed, through molecular biology techniques, into the genome of a host cell. For example, genetic elements can be placed into the chromosomes of the host cell as opposed to in a vector such as a plasmid carried by the host cell. Methods for integrating genetic elements into the genome of a host cell are well known in the art and include homologous recombination.

The heterologous nucleic acid molecule can be present in one or more copies in the yeast host cell’s genome. Alternatively, the heterologous nucleic acid molecule can be independently replicating from the host cell’s genome. In such embodiment, the nucleic acid molecule can be stable and self-replicating.

[0046] In some embodiments, heterologous nucleic acid molecules which can be introduced into the recombinant host cells are codon-optimized with respect to the intended recipient recombinant yeast host cell. As used herein the term “codon-optimized coding region” means a nucleic acid coding region that has been adapted for expression in the cells of a given organism by replacing at least one, or more than one, codons with one or more codons that are more frequently used in the genes of that organism. In general, highly expressed genes in an organism are biased towards codons that are recognized by the most abundant tRNA species in that organism. One measure of this bias is the “codon adaptation index” or “CAI,” which measures the extent to which the codons used to encode each amino acid in a particular gene are those which occur most frequently in a reference set of highly expressed genes from an organism. The CAI of codon optimized heterologous nucleic acid molecule described herein corresponds to between about 0.8 and 1.0, between about 0.8 and 0.9, or about 1.0.

[0047] In some embodiments, heterologous nucleic acid molecules which can be introduced into the recombinant host cells are codon-optimized with respect to the intended recipient recombinant yeast host cell so as to limit or prevent homologous recombination with the corresponding native gene.

[0048] The heterologous nucleic acid molecules of the present disclosure comprise a coding region for the one or more enzymes to be expressed by the host cell. A DNA or RNA “coding region” is a DNA or RNA molecule which is transcribed and/or translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. “Suitable regulatory regions” refer to nucleic acid regions located upstream (5’ non-coding sequences), within, or downstream (3’ non-coding sequences) of a coding region, and which influence the transcription, RNA processing or stability, or translation of the associated coding region.

[0049] Regulatory regions may include promoters, translation leader sequences, RNA processing sites, effector binding sites and stem-loop structures. The boundaries of the coding region are determined by a start codon at the 5’ (amino) terminus and a translation stop codon at the 3’ (carboxyl) terminus. A coding region can include, but is not limited to, prokaryotic regions, cDNA from mRNA, genomic DNA molecules, synthetic DNA molecules, or RNA molecules. If the coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3’ to the coding region. In an embodiment, the coding region can be referred to as an open reading frame. “Open reading frame” is abbreviated ORF and means a length of nucleic acid, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

[0050] The nucleic acid molecules described herein can comprise a non-coding region, for example a transcriptional and/or translational control regions. “Transcriptional and

translational control regions" are DNA regulatory regions, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding region in a host cell. In eukaryotic cells, polyadenylation signals are control regions.

[0051] The heterologous nucleic acid molecule can be introduced in the host cell using a vector. A "vector," e.g., a "plasmid", "cosmid" or "artificial chromosome" (such as, for example, a yeast artificial chromosome) refers to an extra chromosomal element and is usually in the form of a circular double-stranded DNA molecule. Such vectors may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a host cell.

[0052] In the heterologous nucleic acid molecule described herein, the promoter and the nucleic acid molecule coding for the one or more enzymes can be operatively linked to one another. In the context of the present disclosure, the expressions "operatively linked" or "operatively associated" refers to fact that the promoter is physically associated to the nucleotide acid molecule coding for the one or more enzyme in a manner that allows, under certain conditions, for expression of the one or more enzyme from the nucleic acid molecule. In an embodiment, the promoter can be located upstream (5') of the nucleic acid sequence coding for the one or more enzyme. In still another embodiment, the promoter can be located downstream (3') of the nucleic acid sequence coding for the one or more enzyme. In the context of the present disclosure, one or more than one promoter can be included in the heterologous nucleic acid molecule. When more than one promoter is included in the heterologous nucleic acid molecule, each of the promoters is operatively linked to the nucleic acid sequence coding for the one or more enzyme. The promoters can be located, in view of the nucleic acid molecule coding for the one or more protein, upstream, downstream as well as both upstream and downstream.

[0053] "Promoter" refers to a DNA fragment capable of controlling the expression of a coding sequence or functional RNA. The term "expression," as used herein, refers to the transcription and stable accumulation of sense (mRNA) from the heterologous nucleic acid molecule described herein. Expression may also refer to translation of mRNA into a polypeptide. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression at different stages of development, or in response to different environmental or physiological conditions. Promoters which cause a gene to be expressed in most cells at most times at a substantial similar level are commonly referred to as "constitutive promoters". It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity. A promoter is generally bounded at its 3' terminus by the transcription initiation site and extends

upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of the polymerase.

[0054] The promoter can be heterologous to the nucleic acid molecule encoding the one or more enzymes. The promoter can be heterologous or derived from a strain being from the same genus or species as the host cell. In an embodiment, the promoter is derived from the same genus or species of the yeast host cell and the heterologous polypeptide is derived from different genus that the host cell.

[0055] In some embodiments, the present disclosure concerns the expression of one or more heterologous enzyme, a variant thereof or a fragment thereof in a host cell. The enzyme "variants" have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the heterologous enzymes described herein and exhibits the biological activity associated with the heterologous enzyme. In an embodiment, the variant enzyme exhibits at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of the biological activity of the wild-type heterologous enzyme. A variant comprises at least one amino acid difference when compared to the amino acid sequence of the native enzyme. The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. The level of identity can be determined conventionally using known computer programs. Identity can be readily calculated by known methods, including but not limited to those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1993); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Stockton Press, NY (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignments of the sequences disclosed herein were performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PEN ALT Y=10). Default parameters for pairwise alignments using the Clustal method were KTUPLB 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. The variant heterologous enzymes described herein may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another com-

pound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the polypeptide.

[0056] A “variant” of the enzyme can be a conservative variant or an allelic variant. As used herein, a conservative variant refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the enzyme. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the enzyme. For example, the overall charge, structure or hydrophobic-hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the enzyme.

[0057] The heterologous enzyme can be a fragment of an enzyme or fragment of a variant of an enzyme which exhibits the biological activity of the heterologous enzyme or the variant. In an embodiment, the fragment enzyme exhibits at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of the biological activity of the heterologous enzyme or variant thereof. Enzyme “fragments” have at least at least 100, 200, 300, 400, 500 or more consecutive amino acids of the enzyme or the enzyme variant. A fragment comprises at least one less amino acid residue when compared to the amino acid sequence of the enzyme and still possess the enzymatic activity of the full-length enzyme. In some embodiments, the “fragments” have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the enzymes described herein. In some embodiments, fragments of the enzymes can be employed for producing the corresponding full-length enzyme by peptide synthesis. Therefore, the fragments can be employed as intermediates for producing the full-length proteins.

[0058] In some additional embodiments, the present disclosure also provides expressing a protein encoded by a gene ortholog of a gene known to encode an enzyme. A “gene ortholog” is understood to be a gene in a different species that evolved from a common ancestral gene by speciation. In the context of the present invention, a gene ortholog encodes an enzyme exhibiting the same biological function than the native enzyme.

[0059] In some further embodiments, the present disclosure also provides expressing a protein encoded by a gene paralog of a gene known to encode an enzyme. A “gene paralog” is understood to be a gene related by duplication within the genome. In the context of the present invention, a gene paralog encodes an enzyme that could exhibit additional biological function than the native enzyme.

Yeast Host Cell

[0060] In the context of the present disclosure, the combination comprises a yeast host cell which can, in some embodiments, be recombinant. Suitable yeast host cells can be, for example, from the genus *Saccharomyces*, *Kluyveromyces*, *Arxula*, *Debaryomyces*, *Candida*, *Pichia*, *Phaffia*, *Schizosaccharomyces*, *Hansenula*, *Kloeckera*, *Schwanniomyces* or *Yarrowia*. Suitable yeast species can include, for example, *S. cerevisiae*, *S. bulderi*, *S. barnetti*, *S. exiguis*, *S. uvarum*, *S. diastaticus*, *K. lactis*, *K. marxianus* or *K. fragilis*.

In some embodiments, the yeast is selected from the group consisting of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Pichia pastoris*, *Pichia stipitis*, *Yarrowia lipolytica*, *Hansenula polymorpha*, *Phaffia rhodozyma*, *Candida utilis*, *Arxula adeninivorans*, *Debaryomyces hansenii*, *Debaryomyces polymorphus*, *Schizosaccharomyces pombe* and *Schwanniomyces occidentalis*. In one particular embodiment, the yeast is *Saccharomyces cerevisiae*. In some embodiments, the host cell can be an oleaginous yeast cell. For example, the oleaginous yeast host cell can be from the genus *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon* or *Yarrowia*. In some alternative embodiments, the host cell can be an oleaginous microalgae host cell (e.g., for example, from the genus *Thraustochytrium* or *Schizochytrium*). In an embodiment, the yeast host cell is from the genus *Saccharomyces* and, in some embodiments, from the species *Saccharomyces cerevisiae*.

[0061] The yeast host cell of the present disclosure can have a first metabolic pathway comprising one or more enzymes for producing a first metabolic product. The yeast host cell can have the intrinsic ability to produce the first metabolic product or can be engineered to have increased activity in one or more first enzymes in the first metabolic pathway. The increased in activity can be caused at least in part by introducing of one or more first genetic modifications in a native yeast host cell to obtain the recombinant yeast host cell. As such, the activity of the one or more first enzymes of the recombinant yeast host cell is considered “increased” because it is higher than the activity of the one or more first enzymes in the native yeast host cell (e.g., prior to the introduction of the one or more first genetic modifications). The one or more first genetic modifications is not limited to a specific modification provided that it does increase the activity, and in some embodiments, the expression of the one or more first enzymes. For example, the one or more first genetic modifications can include the addition of a promoter to increase the expression of the one or more (endogenous) first enzymes. Alternatively or in addition, the one or more first genetic modifications can include the introduction of one or more copies of a gene(s) encoding the one or more first (heterologous) enzymes in the recombinant yeast host cell.

[0062] In an embodiment, the first metabolic product is a carbohydrate and the yeast host cell has the ability to produce the carbohydrate or has increased activity in one or more first enzymes for producing the carbohydrate. In some embodiments, the first metabolic product is a carbohydrate which is not substantially metabolized by the yeast host cell. For example, the first metabolic product can be a pentose sugars or sugar polymers with a degree of polymerization of 2, 3, 4, or more. Exemplary sugars not naturally or not preferentially utilized by yeasts include, but are not limited to, xylose, arabinose, trehalose, maltose, isomaltose, cellobiose, cellobiotriose, maltotriose, isomaltotriose, panose, raffinose, stachyose, maltotetraose, and maltodextrin. In another embodiment, the first metabolic product can be a sugar alcohol, a 2- to 24-carbon chain including at least one alcohol moiety. Sugar alcohols include, but are not limited to, ethylene glycol, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, galactitol, fucitol, iditol, inositol, volemitol, isomalt, maltitol, lactitol, maltotriitol, maltotetraitol or polyglycitol. In still another embodiment,

the first metabolic product can be an protectant for the yeast host cell, e.g. it has the ability to protect, at least in part, the yeast host of cell from a stressor (lactic acid, formic acid, bacterial contamination, etc.).

[0063] In a specific embodiment, the first metabolic product is trehalose. In such specific embodiment, the yeast host cell can have increased biological activity in at least one of a trehalose-6-phosphate (trehalose-6-P) synthase or a trehalose-6-phosphate phosphatase or both enzymes. As indicated above, this can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous trehalose-6-P synthase and/or the endogenous trehalose-6-P phosphatase. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding an heterologous trehalose-6-P synthase and/or an heterologous trehalose-6-P phosphatase. In an embodiment, the yeast host cell has increased biological activity of a trehalose-6-P synthase, but not of the trehalose-6-P phosphatase. In another embodiment, the yeast host cell has increased biological activity of a trehalose-6-P phosphatase, but not of the trehalose-6-P synthase. In still another embodiment, the yeast host cell has increased biological activity in both a trehalose-6-P synthase and a trehalose-6-P phosphatase.

[0064] As used herein, the term “trehalose-6-phosphate synthase” refers to an enzyme capable of catalyzing the conversion of glucose-6-phosphate and UDP-D-glucose to α - α -trehalose-6-phosphate and UDP. In *Saccharomyces cerevisiae*, the trehalose-6-phosphate synthase gene can be referred to TPS1 (SGD: S000000330, Gene ID: 852423), BYP1, CIF1, FDP1, GGS1, GLC6 or TSS1. The yeast host cell of the present disclosure can include a native gene encoding for the trehalose-6-phosphate synthase and/or an heterologous nucleic acid molecule coding for TPS1, a variant thereof, a fragment thereof or for a protein encoded by a tps1 gene ortholog. In some embodiments, the yeast host cell has an heterologous nucleic acid sequence for the expression of the amino acid sequence of SEQ ID NO: 9, a variant of SEQ ID NO: 9 or a fragment of SEQ ID NO: 9.

[0065] As also used herein, the term “trehalose-6-phosphate phosphatase” refers to an enzyme capable of catalyzing the conversion of α - α -trehalose-6-phosphate and H₂O into phosphate and trehalose. In *Saccharomyces cerevisiae*, the trehalose-6-phosphate phosphatase gene can be referred to TPS2 (SGD: S000002481, Gene ID: 851646), HOG2 or PFK3. The yeast host cell of the present disclosure can include a native gene encoding for the trehalose-6-phosphate phosphatase and/or a nucleic acid molecule coding for TPS2, a variant thereof, a fragment thereof or for a protein encoded by a tps2 gene ortholog. In some embodiments, the yeast host cell has an heterologous nucleic acid sequence for the expression of the amino acid sequence of SEQ ID NO: 10, a variant of SEQ ID NO: 10 or a fragment of SEQ ID NO: 10.

[0066] Alternatively or in combination, the yeast host cell has increased biological activity in a protein involved in regulating trehalose production. As indicated above, this can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous protein involved in regulating trehalose production. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding a protein involved in regulating trehalose production.

[0067] As used herein, the term “protein involved in regulating trehalose production” refers to a protein capable of modulating the activity of enzymes involved in the production of trehalose. In *Saccharomyces cerevisiae*, proteins involved in regulating trehalose production include, but are not limited to a subunit of the trehalose 6-phosphate synthase/phosphatase TPS3 and trehalose synthase long chain (TSL1).

[0068] In some specific embodiments, the protein involved in regulating trehalose production is TSL1. The yeast host cell of the present disclosure can include a native TSL1 protein and/or express an heterologous TSL1 (as well as a variant or a fragment thereof) from any origin including, but not limited to *Saccharomyces cerevisiae* (SGD: S000004566, Gene ID 854872), *Gallus gallus* (Gene ID107050801), *Kluyveromyces marxianus* (Gene ID: 34714558), *Saccharomyces eubayanus* (Gene ID: 28933129), *Schizosaccharomyces japonicus* (Gene ID: 7049746), *Pichia kudriavzevii* (Gene ID: 31691677) or *Hydra vulgaris* (Gene ID 105848257).

[0069] In some additional embodiments (which may be an alternative or a combination to the previous embodiment), the protein involved in regulating trehalose production is TPS3. The yeast host cell of the present disclosure can including a native TPS3 polypeptide and/or express an heterologous TPS3 (as well as a variant or a fragment thereof) from any origin including, but not limited to *Saccharomyces cerevisiae* (SGD: S000004874, Gene ID: 855303), *Arabidopsis thaliana* (Gene ID: 838270), *Sugiyamaella lignohabitans* (Gene ID: 30034940), *Candida albicans* (Gene ID: 3641205), *Chlamydomonas reinhardtii* (Gene ID: 5717648), *Candida orthopsis* (Gene ID: 14539600), *Isaria fumosorosea* (Gene ID: 30022220), *Penicillium digitatum* (Gene ID: 26236600), *Cordyceps militaris* (Gene ID: 18168860), *Aspergillus fumigatus* (Gene ID: 3506432), *Aspergillus flavus* (Gene ID: 7918663), *Aspergillus clavatus* (Gene ID: 4705657), *Aspergillus fischeri* (Gene ID: 4588220), *Aspergillus vadensis* (Gene ID 37209217), *Aspergillus costaricensis* (Gene ID: 37185236), *Aspergillus piperis* (Gene ID: 37160157), *Aspergillus aculeatinus* (Gene ID: 37150689), *Aspergillus neoniger* (Gene ID: 37124414), *Aspergillus sclerotioriniger* (Gene ID: 37114541), *Aspergillus brunneoviolaceus* (Gene ID: 37089207), *Aspergillus saccharolyticus* (Gene ID: 37076724), *Aspergillus eucaalypticola* (Gene ID: 37051636), *Aspergillus novofumigatus* (Gene ID: 36535454), *Verticillium dahliae* (Gene ID: 20704316), *Trichophyton rubrum* (Gene ID: 10373473), *Nannizia gypsea* (Gene ID: 10027518), *Verticillium alfalfae* (Gene ID: 9532751), *Ajellomyces dermatitidis* (Gene ID: 8508720), *Talaromyces stipitatus* (Gene ID: 8104915) or *Talaromyces marneffei* (Gene ID: 7024067).

[0070] In some embodiments, especially when the metabolism of the first metabolic product is oxidative (for example when it is mannitol, sorbitol or glycerol), the present disclosure provides a yeast host cell which can be genetically modified to provide a secondary substrate to the bacterial host cell which could act as an electron acceptor and allow redox balance. This can be done, for example, by introducing one or more heterologous nucleic acid molecules encoding a NADP⁺-dependent aldehyde dehydrogenase and/or a phosphoketolase. This can also be done by introducing a strong promoter upstream of the native NADP⁺-dependent aldehyde dehydrogenase and/or phosphoketolase to increase its level of expression. Alternatively

or in combination, this can be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding a protein having NADP⁺-dependent aldehyde dehydrogenase and/or phosphoketolase activity. The adjustment of the redox balance can also be done, alternatively or in combination, by supplementing the fermentation medium with an electron acceptor, such as, for example acetate.

[0071] As used in the context of the present disclosure, the NADP⁺-dependent aldehyde dehydrogenase is an enzyme that catalyzes the conversion of an aldehyde, NADP⁺ and water into an acid, NADPH and an hydrogen atom (E.C. 1.2.1.4). In an embodiment, the NADP⁺-dependent aldehyde dehydrogenase can be derived from *S. cerevisiae* ALD6 (Gene ID: 856044), *Candida albicans* ALD6 (Gene ID: 3647407), *Kluyveromyces marxianus* ALD6 (Gene ID: 34714396) or *Candida orthopsisilosis* (Gene ID: 14538090).

[0072] As used in the context of the present disclosure, the phosphoketolase (PHK) is an enzyme that catalyzes D-xylulose 5-phosphate and phosphate into acetyl phosphate, D-glyceraldehyde 3-phosphate and water (E.C. 4.1.2.9 and 4.1.2.22). In some embodiments, PHK is up-regulated. In some embodiments, single-specificity phosphoketolase is up-regulated. In some embodiments, dual-specificity phosphoketolase is up-regulated. In some embodiments, the PHK is derived from a genus selected from the group consisting of *Aspergillus*, *Neurospora*, *Lactobacillus*, *Bifidobacterium*, and *Penicillium*. In some embodiments, the PHK is from *Bifidobacterium adolescentis*. In some embodiments the PHK is from *Aspergillus niger*. In some embodiments, the PHK is from *Neurospora crassa*. In some embodiments, the PHK is from *Lactobacillus paracasei*. In some embodiments, the PHK is from *Lactobacillus plantarum*.

[0073] In another specific embodiment, the first metabolic product is a carbohydrate, which is a sugar alcohol and in some specific embodiments, the carbohydrate is mannitol. In such embodiment, the yeast host cell can have native mannitol dehydrogenase activity and/or be genetically modified to increased mannitol dehydrogenase activity. In an embodiment, the mannitol dehydrogenase activity is provided by the enzyme mannitol-1-phosphate 5-dehydrogenase catalyzes the conversion of fructose-6-phosphate and NADH into mannitol-1-phosphate and NAD⁺ (EC 1.1.1.17). Mannitol-1-phosphate can then be converted to mannitol via the promiscuous phosphatase activity of the yeast host cell. Alternatively or in combination, the yeast host cell can have native mannitol 1-phosphate phosphatase activity and/or can be engineered to provide or increase mannitol 1-phosphate phosphatase activity. As indicated above, the increase in mannitol-1-phosphate 5-dehydrogenase activity can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous mannitol-1-phosphate 5-dehydrogenase. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding mannitol-1-phosphate 5-dehydrogenase. The mannitol-1-phosphate 5-dehydrogenase can be derived from the mtID gene. The mtID gene encoding the mannitol-1-phosphate 5-dehydrogenase can be of yeast or bacterial origin. In some embodiments, the mtID is derived from a genus selected from the group consisting of *Escherichia*, *Aspergillus*, *Neurospora*, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Bacillus*, and *Acinetobacter*. In some embodiments, mtID is up-regulated. In some embodiments, the mtID is from

Escherichia coli. In some embodiments the mtID is from *Lactobacillus paracasei*. In some embodiments, the mtID is from *Lactobacillus plantarum*. In some embodiments, the mtID is from *Lactococcus lactis*. In some embodiments, the mtID is from *Bacillus subtilis*. In some embodiments the mtID is from *Pseudomonas* sp. In some embodiments the mtID is from *Acinetobacter baylyi*. In some embodiments the mtID is from *Aspergillus niger*. In an embodiment, the MTLD polypeptide is from *Escherichia* sp., such as, for example *Escherichia coli*. In such embodiment, the MTLD polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 27, be a variant of the amino acid sequence of SEQ ID NO: 27 or be a fragment of the amino acid sequence of SEQ ID NO: 27 or a variant thereof. In an embodiment, the MTLD polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 28, being a variant of the nucleic acid sequence of SEQ ID NO: 28 or being a fragment of the nucleic acid sequence or SEQ ID NO: 28 or a variant thereof. In an embodiment, the MTLD2 polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the MTLD2 polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 39, be a variant of the amino acid sequence of SEQ ID NO: 39 or be a fragment of the amino acid sequence of SEQ ID NO: 39 or a variant thereof. In an embodiment, the MTLD2 polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 40, being a variant of the nucleic acid sequence of SEQ ID NO: 40 or being a fragment of the nucleic acid sequence or SEQ ID NO: 40 or a variant thereof.

[0074] In another specific embodiment, the carbohydrate is a sugar alcohol and in some specific embodiments, the carbohydrate is sorbitol. In such embodiment, the yeast host cell can have native sorbitol dehydrogenase activity and/or can be modified to provide or increase sorbitol dehydrogenase activity. In an embodiment, the sorbitol dehydrogenase activity is provided by the enzyme sorbitol-6-phosphate 2-dehydrogenase which catalyzes the conversion of fructose-6-phosphate and NADH into sorbitol 6-phosphate and NAD⁺ (EC 1.1.1.140). Sorbitol 6-phosphate can then be converted to sorbitol via the promiscuous phosphatase activity of the yeast host cell. Alternatively or in combination, the yeast host cell can have native sorbitol-6-phosphate phosphatase activity and/or be genetically modified to provide or increase sorbitol-6-phosphate phosphatase activity. As indicated above, the increase in sorbitol 6-phosphate 2-dehydrogenase activity can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous sorbitol 6-phosphate 2-dehydrogenase. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding sorbitol 6-phosphate 2-dehydrogenase. The gene encoding the sorbitol 6-phosphate 2-dehydrogenase can be of yeast or bacterial origin. In an embodiment, the sorbitol 6-phosphate 2-dehydrogenase can be encoded by the srlD gene. In some embodiments, the srlD is derived from a genus selected from the group consisting of *Escherichia*, *Lactobacillus*, *Clostridium*, *Streptococcus*, and *Klebsiella*. In some embodiments, the srlD gene is up-regulated. In some embodiments, the srlD gene is from *Escherichia coli*. In some embodiments the srlD gene is from *Lactobacillus paracasei*. In some embodiments, the srlD gene is from *Lactobacillus plantarum*. In some embodi-

ments the srlD gene is from *Clostridium pasteurianum*. In some embodiments the srlD gene is from *Klebsiella aerogenes*. The gene encoding the sorbitol 6-phosphate dehydrogenase can be derived from the srlD gene and can be, without limitations, from the following sources: *Escherichia coli* (Gene ID: 948937), *Clostridioides difficile* (4915542), *Mycoplasma mycoides* subsp. *mycoides* (Gene ID: 2744550), *Clostridium botulinum* (Gene ID: 5399122), *Shigella dysenteriae* (Gene ID: 3796629), *Shigella flexneri* (Gene ID: 1027455), *Escherichia coli* (Gene ID: 7152897 or 7157974), *Salmonella enterica* subsp. *enterica* (Gene ID: 1254358 or 1249263), *Clostridium botulinum* (Gene ID: 5187667) or *Saccharomyces cerevisiae* (Gene IDs: 851539 and 854095). In an embodiment, the SRLD polypeptide is from *Escherichia* sp., such as, for example *Escherichia coli*. In such embodiment, the SRLD polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 29, be a variant of the amino acid sequence of SEQ ID NO: 29 or be a fragment of the amino acid sequence of SEQ ID NO: 29 or a variant thereof. In an embodiment, the SRLD polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 30, being a variant of the nucleic acid sequence of SEQ ID NO: 30 or being a fragment of the nucleic acid sequence of SEQ ID NO: 30 or a variant thereof.

[0075] In another specific embodiment, the carbohydrate is a sugar alcohol and in some specific embodiments, the carbohydrate is glycerol. In such embodiment, the yeast host cell does not need to be genetically modified as it has the intrinsic ability to produce glycerol. Alternatively, the yeast host cell can be genetically modified to increase dihydrogenaseacetone-3-phosphate dehydrogenase activity and/or glycerol-phosphate phosphatase activity.

[0076] The yeast host cell of the present disclosure can have a second metabolic pathway comprising one or more enzymes for producing a second metabolic product. The yeast host cell can have the intrinsic ability to produce the second metabolic product or can be engineered to have increased activity in one or more second enzymes in the second metabolic pathway. The increased in activity can be caused at least in part to the introduction of one or more second genetic modifications in a native yeast host cell to obtain the recombinant yeast host cell. As such, the activity of the one or more second enzymes of the recombinant yeast host cell is considered "increased" because it is higher than the activity of the one or more second enzymes in the native yeast host cell (e.g., prior to the introduction of the one or more second genetic modifications). The one or more second genetic modifications is not limited to a specific modification provided that it does increase the activity, and in some embodiments, the expression of the one or more second enzymes. For example, the one or more second genetic modifications can include the addition of a promoter to increase the expression of the one or more (endogenous) second enzymes. Alternatively or in addition, the one or more second genetic modifications can include the introduction of one or more copies of a gene(s) encoding the one or more second (heterologous) enzymes in the recombinant yeast host cell.

[0077] In an embodiment, the second metabolic product is ethanol and the yeast host cell has the ability to produce the ethanol from the organic acid (or associated ester) or has increased activity in one or more second enzymes for converting the organic acid into ethanol. In an embodiment,

the organic acid can be, without limitation, acetic acid. As used in the context of the present disclosure, the expression "organic acid" includes associated organic esters which can be hydrolyzed into the organic acid. An embodiment of an organic acid is acetic acid and an embodiment of a corresponding organic ester is acetate.

[0078] In a specific embodiment in which the yeast host cell is capable of converting the organic acid (or associated ester) into ethanol, the yeast host cell can have increased biological activity in a polypeptide having acetylating aldehyde dehydrogenase activity. As used in the present disclosure, a polypeptide having acetylating aldehyde dehydrogenase activity has the ability to convert acetyl-coA into an aldehyde. In some embodiments, the polypeptide having acetylating aldehyde dehydrogenase activity is an AADH or is a bifunctional acetylating aldehyde dehydrogenase/alcohol dehydrogenase (ADHE). The bifunctional acetaldehyde/alcohol dehydrogenase is an enzyme capable of converting acetyl-CoA into acetaldehyde as well as acetaldehyde into ethanol. Heterologous bifunctional acetaldehyde/alcohol dehydrogenases (AADH) include but are not limited to those described in U.S. Pat. No. 8,956,851 and WO 2015/023989. Heterologous AADHs of the present disclosure include, but are not limited to, the ADHE polypeptides or a polypeptide encoded by an adhe gene ortholog. In an embodiment, the AADH is from a *Bifidobacterium* sp., such as for example, a *Bifidobacterium adolescentis*. In an embodiment, the AADH has the amino acid sequence of SEQ ID NO: 15 or 47, is a variant of the amino acid sequence of SEQ ID NO: 15 or 47 or is a fragment of the amino acid sequence of SEQ ID NO: 15 or 47. In such embodiment, the genetic modification can comprise introducing an heterologous nucleic acid molecule (which can have, in some embodiments, the nucleic acid sequence of SEQ ID NO: 48) encoding a protein having the amino acid sequence of SEQ ID NO: 15 or 47, being a variant of the amino acid sequence of SEQ ID NO: 15 or 47 or being a fragment of the amino acid sequence of SEQ ID NO: 15 or 47. In a specific embodiment in which the yeast host cell is capable of converting the organic acid (such as, for example acetic acid or its associated ester acetate) into ethanol, the yeast host cell can have increased biological activity in an acetyl-coA synthetase. The acetyl-coA synthetase is an enzyme capable of converting acetic acid into acetyl-CoA. Heterologous acetyl-coA synthetase include but are not limited to GenBank Accession number CAA97725. Heterologous acetyl-coA synthetase of the present disclosure include, but are not limited to, the ACS2 polypeptides or a polypeptide encoded by an acs2 gene ortholog. In an embodiment, the AADH (e.g., ACS2) is from a *Saccharomyces* sp., such as for example, a *Saccharomyces cerevisiae*. In an embodiment, the acetyl-coA synthetase has the amino acid sequence of SEQ ID NO: 49, is a variant of the amino acid sequence of SEQ ID NO: 49 or is a fragment of the amino acid sequence of SEQ ID NO: 49. In such embodiment, the genetic modification can comprise introducing an heterologous nucleic acid molecule encoding a protein having the amino acid sequence of SEQ ID NO: 50, being a variant of the amino acid sequence of SEQ ID NO: 50 or being a fragment of the amino acid sequence of SEQ ID NO: 50.

[0079] In a specific embodiment in which the yeast host cell is capable of converting the organic acid (such as, for example acetic acid or its associated ester acetate) into ethanol, the yeast host cell can have increased biological

activity in a NADPH-dependent alcohol dehydrogenase. The protein having NADPH-dependent alcohol dehydrogenase activity can be an ADH polypeptide (for example from *Entamoeba* sp., including *Entamoeba nuttalli* (such as, for example, the one having the amino acid sequence of SEQ ID NO: 45), an ADH1 polypeptide variant (e.g., a variant of the amino acid sequence of SEQ ID NO: 45), an ADH1 polypeptide fragment (e.g., a fragment of the amino acid sequence of SEQ ID NO: 45 or a variant thereof) or a polypeptide encoded by an adhl gene ortholog/paralog. In such embodiment, the genetic modification can comprise introducing an heterologous nucleic acid molecule encoding a protein having the amino acid sequence of SEQ ID NO: 46, being a variant of the amino acid sequence of SEQ ID NO: 46 or being a fragment of the amino acid sequence of SEQ ID NO: 46.

[0080] In some embodiments, the recombinant yeast host cell can also include one or more additional genetic modifications limiting the production of glycerol. For example, the additional genetic modification can be a genetic modification leading to the reduction in the production, and in an embodiment to the inhibition in the production, of one or more native enzymes that function to produce glycerol. As used in the context of the present disclosure, the expression “reducing the production of one or more native enzymes that function to produce glycerol” refers to a genetic modification which limits or impedes the expression of genes associated with one or more native polypeptides (in some embodiments enzymes) that function to produce glycerol, when compared to a corresponding yeast strain which does not bear such genetic modification. In some instances, the additional genetic modification reduces but still allows the production of one or more native polypeptides that function to produce glycerol. In other instances, the genetic modification inhibits the production of one or more native enzymes that function to produce glycerol. Polypeptides that function to produce glycerol refer to polypeptides which are endogenously found in the recombinant yeast host cell. Native enzymes that function to produce glycerol include, but are not limited to, the GPD1 and the GPD2 polypeptide (also referred to as GPD1 and GPD2, respectively) as well as the GPP1 and the GPP2 polypeptides (also referred to as GPP1 and GPP2, respectively). In an embodiment, the recombinant yeast host cell bears a genetic modification in at least one of the gpd1 gene (encoding the GPD1 polypeptide), the gpd2 gene (encoding the GPD2 polypeptide), the gpp1 gene (encoding the GPP1 polypeptide) or the gpp2 gene (encoding the GPP2 polypeptide). In another embodiment, the recombinant yeast host cell bears a genetic modification in at least two of the gpd1 gene (encoding the GPD1 polypeptide), the gpd2 gene (encoding the GPD2 polypeptide), the gpp1 gene (encoding the GPP1 polypeptide) or the gpp2 gene (encoding the GPP2 polypeptide). Examples of recombinant yeast host cells bearing such genetic modification(s) leading to the reduction in the production of one or more native enzymes that function to produce glycerol are described in WO 2012/138942. In some embodiments, the recombinant yeast host cell has a genetic modification (such as a genetic deletion or insertion) only in one enzyme that functions to produce glycerol, in the gpd2 gene, which would cause the host cell to have a knocked-out gpd2 gene. In some embodiments, the recombinant yeast host cell can have a genetic modification in the gpd1 gene and the gpd2 gene resulting in a recombinant yeast host cell being knock-

out for the gpd1 gene and the gpd2 gene. In some specific embodiments, the recombinant yeast host cell can have a knock-out for the gpd1 gene and have duplicate copies of the gpd2 gene (in some embodiments, under the control of the gpd1 promoter). In still another embodiment (in combination or alternative to the genetic modification described above).

[0081] In yet another embodiment, the recombinant yeast host cell does not bear an additional genetic modification and includes its native genes coding for the GPP/GDP proteins. As such, in some embodiments, there are no genetic modifications leading to the reduction in the production of one or more native enzymes that function to produce glycerol in the recombinant yeast host cell.

[0082] Alternatively or in combination, the recombinant yeast host cell can also include one or more additional genetic modifications facilitating the transport of glycerol in the recombinant yeast host cell. For example, the additional genetic modification can be a genetic modification leading to the increase in activity of one or more native enzymes that function to transport glycerol. Native enzymes that function to transport glycerol synthesis include, but are not limited to, the FPS1 polypeptide as well as the STL1 polypeptide. The FPS1 polypeptide is a glycerol exporter and the STL1 polypeptide functions to import glycerol in the recombinant yeast host cell. By either reducing or inhibiting the expression of the FPS1 polypeptide and/or increasing the expression of the STL1 polypeptide, it is possible to control, to some extent, glycerol synthesis. The STL1 protein is natively expressed in yeasts and fungi, therefore the heterologous protein functioning to import glycerol can be derived from yeasts and fungi. STL1 genes encoding the STL1 protein include, but are not limited to, *Saccharomyces cerevisiae* Gene ID: 852149, *Candida albicans*, *Kluyveromyces lactis* Gene ID: 2896463, *Ashbya gossypii* Gene ID: 4620396, *Eremothecium sinecaudum* Gene ID: 28724161, *Torulaspora delbrueckii* Gene ID: 11505245, *Lachancea thermotolerans* Gene ID: 8290820, *Phialophora attae* Gene ID: 28742143, *Penicillium digitatum* Gene ID: 26229435, *Aspergillus oryzae* Gene ID: 5997623, *Aspergillus fumigatus* Gene ID: 3504696, *Talaromyces atroroseus* Gene ID: 31007540, *Rasamonia emersonii* Gene ID: 25315795, *Aspergillus flavus* Gene ID: 7910112, *Aspergillus terreus* Gene ID: 4322759, *Penicillium chrysogenum* Gene ID: 8310605, *Alternaria alternata* Gene ID: 29120952, *Paraphaeosphaeria sporulosa* Gene ID: 28767590, *Pyrenophaora tritici-repentis* Gene ID: 6350281, *Metarhizium robertsii* Gene ID: 19259252, *Isaria fumosorosea* Gene ID: 30023973, *Cordyceps militaris* Gene ID: 18171218, *Pochonia chlamydosporia* Gene ID: 28856912, *Metarhizium majus* Gene ID: 26274087, *Neofusicoccum parvum* Gene ID: 19029314, *Diplodia corticola* Gene ID: 31017281, *Verticillium dahliae* Gene ID: 20711921, *Colletotrichum gloeosporioides* Gene ID: 18740172, *Verticillium albo-atrum* Gene ID: 9537052, *Paracoccidioides lutzii* Gene ID: 9094964, *Trichophyton rubrum* Gene ID: 10373998, *Nannizia gypsea* Gene ID: 10032882, *Trichophyton verrucosum* Gene ID: 9577427, *Arthroderma benhamiae* Gene ID: 9523991, *Magnaporthe oryzae* Gene ID: 2678012, *Gaeumannomyces graminis* var. *tritici* Gene ID: 20349750, *Togninia minima* Gene ID: 19329524, *Eutypa lata* Gene ID: 19232829, *Scedosporium apiospermum* Gene ID: 27721841, *Aureobasidium namibiae* Gene ID: 25414329, *Sphaerulina musiva* Gene ID: 27905328 as well as *Pachy-*

solen tannophilus GenBank Accession Numbers JQ481633 and JQ481634, *Saccharomyces paradoxus* STL1 and *Pichia sorbitophilia*. In an embodiment, the STL1 protein is encoded by *Saccharomyces cerevisiae* Gene ID: 852149. The STL1 protein can have the amino acid sequence of SEQ ID NO: 11 or 53, be a variant of the amino acid sequence of SEQ ID NO: 11 or 53 be a fragment of the amino acid sequence of SEQ ID NO: 11 or 53. In still another embodiment, the STL1 protein can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 54, a variant of the nucleic acid sequence of SEQ ID NO: 54 or a fragment of the nucleic acid sequence of SEQ ID NO: 54. In another embodiment, the STL1 protein is encoded by the heterologous STL1 gene of *Pichia sorbitophilia* (also referred to as *Millerzyma farinose*). The STL1 protein can have the amino acid sequence of SEQ ID NO: 51, be a variant of the amino acid sequence of SEQ ID NO: 51 or be a fragment of the amino acid sequence of SEQ ID NO: 51. In still another embodiment, the STL1 protein can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 52, a variant of the nucleic acid sequence of SEQ ID NO: 52 or a fragment of the nucleic acid sequence of SEQ ID NO: 52.

[0083] In some embodiments, the yeast host cell can have a further genetic modification allowing the expression of heterologous NADP-specific alcohol dehydrogenase. The presence of this enzyme increases the availability of cytosolic NADH, by creating a redox imbalance between glycolysis and ethanol fermentation, and increases acetate conversion in the yeast host cell. In an embodiment, the NADP-specific alcohol dehydrogenase is from *Entamoeba* sp., for example from *Entamoeba nuttalli*. In yet another embodiment, the NADP-specific alcohol dehydrogenase has the amino acid sequence of SEQ ID NO: 45, is a variant of the amino acid sequence of SEQ ID NO: 45 or is a fragment of the amino acid sequence of SEQ ID NO: 45. In still another specific embodiment, the NADP-specific alcohol dehydrogenase is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 46, a variant of the nucleic acid sequence of SEQ ID NO: 46 or is a fragment of the nucleic acid sequence of SEQ ID NO: 46.

[0084] Alternatively or in combination, the yeast host cell can have a genetic modification allowing the expression of an heterologous saccharolytic enzyme. As used in the context of the present disclosure, a “saccharolytic enzyme” can be any enzyme involved in carbohydrate digestion, metabolism and/or hydrolysis, including amylases, cellulases, hemicellulases, cellulolytic and amyloytic accessory enzymes, inulinases, levanases, and pentose sugar utilizing enzymes. Amyloytic enzyme. In an embodiment, the saccharolytic enzyme is an amyloytic enzyme. As used herein, the expression “amyloytic enzyme” refers to a class of enzymes capable of hydrolyzing starch or hydrolyzed starch. Amyloytic enzymes include, but are not limited to alpha-amylases (EC 3.2.1.1, sometimes referred to fungal alpha-amylase, see below), maltogenic amylase (EC 3.2.1.133), glucoamylase (EC 3.2.1.3), glucan 1,4-alpha-maltotetrahydrolase (EC 3.2.1.60), pullulanase (EC 3.2.1.41), iso-amylase (EC 3.2.1.68) and amylo maltase (EC 2.4.1.25). In an embodiment, the one or more amyloytic enzymes can be an alpha-amylase from *Aspergillus oryzae*, a maltogenic alpha-amylase from *Geobacillus stearothermophilus*, a glucoamylase from *Saccharomyces fibuligera*, a glucan 1,4-alpha-

maltotetrahydrolase from *Pseudomonas saccharophila*, a pullulanase from *Bacillus naganensis*, a pullulanase from *Bacillus acidopullulyticus*, an iso-amylase from *Pseudomonas amyloborosa*, and/or amylo maltase from *Thermus thermophilus*. Some amyloytic enzymes have been described in WO2018/167670 and are incorporated herein by reference.

[0085] For example, the yeast host cell can bear one or more genetic modifications allowing for the production of an heterologous glucoamylase. Many microbes produce an amylase to degrade extracellular starches. In addition to cleaving the last α (1-4) glycosidic linkages at the non-reducing end of amylose and amylopectin, yielding glucose, γ -amylase will cleave α (1-6) glycosidic linkages. The heterologous glucoamylase can be derived from any organism. In an embodiment, the heterologous protein is derived from a γ -amylase, such as, for example, the glucoamylase of *Saccharomyces fibuligera* (e.g., encoded by the glu 0111 gene). Examples of yeast host cells bearing such second genetic modifications are described in WO 2011/153516 as well as in WO 2017/037614 and herewith incorporated in its entirety. In an embodiment, the yeast host cell can be modified to express an heterologous glucoamylase having the amino acid sequence of SEQ ID NO: 16, a variant thereof or a fragment thereof.

[0086] Alternatively or in combination, the yeast host cell can bear one or more genetic modifications for increasing formate/acetyl-CoA production. In order to do so, yeast host cell can bear one or more genetic modification for increasing its pyruvate formate lyase activity. As used in the context of the present disclosure, “an heterologous enzyme that function to increase formate/acetyl-CoA production” refers to polypeptides which may or may not be endogenously found in the yeast host cell and that are purposefully introduced into the yeast host cells to anabolize formate. In some embodiments, the heterologous enzyme that can be an heterologous pyruvate formate lyase (PFL), such as PFLA or PFLB Heterologous PFL of the present disclosure include, but are not limited to, the PFLA polypeptide, a polypeptide encoded by a pfla gene ortholog, the PFLB polypeptide or a polypeptide encoded by a pflb gene ortholog.

[0087] Embodiments of the pyruvate formate lyase activating enzyme and of PFLA can be derived, without limitation, from the following (the number in brackets correspond to the Gene ID number): *Escherichia coli* (MG1655945517), *Shewanella oneidensis* (1706020), *Bifidobacterium longum* (1022452), *Mycobacterium bovis* (32287203), *Haemophilus parasuis* (7277998), *Mannheimia haemolytica* (15341817), *Vibrio vulnificus* (33955434), *Cronobacter sakazakii* (29456271), *Vibrio alginolyticus* (31649536), *Pasteurella multocida* (29388611), *Aggregatibacter actinomycetemcomitans* (31673701), *Actinobacillus suis* (34291363), *Finegoldia magna* (34165045), *Zymomonas mobilis* subsp. *mobilis* (3073423), *Vibrio tubiashii* (23444968), *Gallibacterium anatis* (10563639), *Actinobacillus pleuropneumoniae* serovar (4849949), *Ruminoclostridium thermocellum* (35805539), *Cylindrospermopsis raciborskii* (34474378), *Lactococcus garvieae* (34204939), *Bacillus cytotoxicus* (33895780), *Providencia stuartii* (31518098), *Pantoea ananatis* (31510290), *Teredinibacter turnerae* (29648846), *Morganella morganii* subsp. *morganii* (14670737), *Vibrio anguillarum* (77510775106), *Dickeya dadantii* (39379733484), *Xenorhabdus bovinii* (8830449), *Edwardsiella ictaluri* (7959196), *Proteus mirabilis*

(6801040), *Rahnella aquatilis* (34350771), *Bacillus pseudomycoides* (34214771), *Vibrio alginolyticus* (29867350), *Vibrio nigripulchritudo* (29462895), *Vibrio orientalis* (25689084), *Kosakonia sacchari* (23844195), *Serratia marcescens* subsp. *marcescens* (23387394), *Shewanella baltica* (11772864), *Vibrio vulnificus* (2625152), *Streptomyces acidiscabies* (33082227), *Streptomyces davaonensis* (31227069), *Streptomyces scabiei* (24308152), *Volvox carteri* f. *nagariensis* (9616877), *Vibrio breogamii* (35839746), *Vibrio mediterranei* (34766273), *Fibrobacter succinogenes* subsp. *succinogenes* (34755395), *Enterococcus gilvus* (34360882), *Akkermansia muciniphila* (34173806), *Enterobacter hormaechei* subsp. *Steigerwaltii* (34153767), *Dickeya zeae* (33924935), *Enterobacter* sp. (32442159), *Serratia odorifera* (31794665), *Vibrio crassostreae* (31641425), *Selenomonas ruminantium* subsp. *lactilytica* (31522409), *Fusobacterium necrophorum* subsp. *funduliforme* (31520833), *Bacteroides uniformis* (31507008), *Haemophilus somnis* (233631487328), *Rodentibacter pneumotropicus* (31211548), *Pectobacterium carotovorum* subsp. *carotovorum* (29706463), *Eikenella corrodens* (29689753), *Bacillus thuringiensis* (29685036), *Streptomyces rimosus* subsp. *Rimosus* (29531909), *Vibrio fluvialis* (29387180), *Leibesia oxytoca* (29377541), *Paragebacillus thermoglucosidans* (29237437), *Aeromonas veronii* (28678409), *Clostridium innocuum* (26150741), *Neisseria mucosa* (25047077), *Citrobacter freundii* (23337507), *Clostridium bolteae* (23114831), *Vibrio tasmaniensis* (7160642), *Aeromonas salmonicida* subsp. *salmonicida* (4995006), *Escherichia coli* 0157: H7 str. *Sakai* (917728), *Escherichia coli* 083: H1 str. (12877392), *Yersinia pestis* (11742220), *Clostridioides difficile* (4915332), *Vibrio fischeri* (3278678), *Vibrio parahaemolyticus* (1188496), *Vibrio corallilyticus* (29561946), *Kosakonia cowanii* (35808238), *Yersinia ruckeri* (29469535), *Gardnerella vaginalis* (99041930), *Listeria fleischmannii* subsp. *Coloradensis* (34329629), *Photobacterium kishitanii* (31588205), *Aggregatibacter actinomycetemcomitans* (29932581), *Bacteroides caccae* (36116123), *Vibrio toranzoniae* (34373279), *Providencia alcalifaciens* (34346411), *Edwardsiella arguillarum* (33937991), *Lonsdalea quercina* subsp. *Quercina* (33074607), *Pantoea septica* (32455521), *Butyrivibrio proteo-clasticus* (31781353), *Photorhabdus temperata* subsp. *Thracensis* (29598129), *Dickeya solani* (23246485), *Aeromonas hydrophila* subsp. *hydrophila* (4489195), *Vibrio cholerae* 01 biovar El Tor str. (2613623), *Serratia rubidaea* (32372861), *Vibrio bivalvicia* (32079218), *Serratia liquefaciens* (29904481), *Gilliamella apicola* (29851437), *Pluralibacter gergoviae* (29488654), *Escherichia coli* 0104: H4 (13701423), *Enterobacter aerogenes* (10793245), *Escherichia coli* (7152373), *Vibrio campbellii* (5555486), *Shigella dysenteriae* (3795967), *Bacillus thuringiensis* serovar *konukian* (2854507), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (1252488), *Bacillus anthracis* (1087733), *Shigella flexneri* (1023839), *Streptomyces griseoruber* (32320335), *Ruminococcus gnavus* (35895414), *Aeromonas fluvalis* (35843699), *Streptomyces ossamyceticus* (35815915), *Xenorhabdus doucetiae* (34866557), *Lactococcus piscium* (34864314), *Bacillus glycinifermentans* (34773640), *Photobacterium damselae* subsp. *Damselae* 34509297, *Streptomyces venezuelae* 34035779, *Shewanella algae* (34011413), *Neisseria sicca* *multitudinisentens* (32575347), *Kitasatospora purpoeufusca* (32375714), *Serratia fonticola* (32345867), *Aeromonas enteropelogenes* (32325051), *Micromonospora aurantiaca* (32162988), *Moritella viscosa* (31933483), *Yersinia aldovae* (31912331), *Leclercia adecarboxylata* (31868528), *Salinivibrio costicola* subsp. *costicola* (31850688), *Aggregatibacter aphrophilus* (31611082), *Photobacterium leiognathi* (31590325), *Streptomyces canus* (31293262), *Pantoea dispersa* (29923491), *Pantoea rwandensis* (29806428), *Paenibacillus borealis* (29548601), *Aliivibrio wodanis* (28541257), *Streptomyces virginiae* (23221817), *Escherichia coli* (7158493), *Mycobacterium tuberculosis* (887973), *Streptococcus mutans* (1028925), *Streptococcus cristatus* (29901602), *Enterococcus hirae* (13176624), *Bacillus licheniformis* (3031413), *Chromobacterium violaceum* (24949178), *Parabacteroides distasonis* (5308542), *Bacteroides vulgatus* (5303840), *Faecalibacterium prausnitzii* (34753201), *Melissococcus plutonius* (34410474), *Streptococcus galloyticus* subsp. *galloyticus* (34397064), *Enterococcus malodoratus* (34355146), *Bacteroides oleiciplenus* (32503668), *Listeria* (985766), *Enterococcus faecalis* (1200510), *Campylobacter jejuni* subsp. *jejuni* (905864), *Lactobacillus plantarum* (1063963), *Yersinia enterocolitica* subsp. *enterocolitica* (4713333), *Streptococcus equinus* (33961143), *Macrococcus canis* (35294771), *Streptococcus sanguinis* (4807186), *Lactobacillus salivarius* (3978441), *Lactococcus lactis* subsp. *lactis* (1115478), *Enterococcus faecium* (12999835), *Clostridium botulinum* A (5184387), *Clostridium acetobutylicum* (1117164), *Bacillus thuringiensis* serovar *konukian* (2857050), *Cryobacterium flavum* (35899117), *Enterovibrio norvegicus* (35871749), *Bacillus acidiceler* (34874556), *Prevotella intermedia* (34516987), *Pseudobutyrivibrio ruminis* (34419801), *Pseudovibrio asciadiaceicola* (34149433), *Corynebacterium coyleae* (34026109), *Lactobacillus curvatus* (33994172), *Cellulosimicrobium cellulans* (33980622), *Lactobacillus agilis* (33975995), *Lactobacillus sakei* (33973512), *Staphylococcus simulans* (32051953), *Obesumbacterium proteus* (29501324), *Salmonella enterica* subsp. *enterica* serovar *Typhi* (1247402), *Streptococcus agalactiae* (1014207), *Streptococcus agalactiae* (1013114), *Legionella pneumophila* subsp. *pneumophila* str. *Philadelphia* (119832735), *Pyrococcus furiosus* (1468475), *Mannheimia haemolytica* (15340992), *Thalassiosira pseudonana* (7444511), *Thalassiosira pseudonana* (7444510), *Streptococcus thermophilus* (31940129), *Sulfolobus solfataricus* (1454925), *Streptococcus iniae* (35765828), *Streptococcus iniae* (35764800), *Bifidobacterium thermophilum* (31839084), *Bifidobacterium animalis* subsp. *lactis* (29695452), *Streptobacillus moniliiformis* (29673299), *Thermoglaadius calderae* (13013001), *Streptococcus oralis* subsp. *tigurinus* (31538096), *Lactobacillus ruminis* (29802671), *Streptococcus parauberis* (29752557), *Bacteroides ovatus* (29454036), *Streptococcus gordonii* str. *Challis* substr. CHI (25052319), *Clostridium botulinum* B str. *Eklund 17B* (19963260), *Thermococcus litoralis* (16548368), *Archaeoglobus sulfaticallidus* (15392443), *Ferroglobus placidus* (8778929), *Archaeoglobus profundus* (8739370), *Listeria seeligeri* serovar 1/2b (32488230), *Bacillus thuringiensis* (31632063), *Rhodobacter capsulatus* (31491679), *Clostridium perfringens* (29571530), *Lactococcus garvieae* (12478921), *Proteus mirabilis* (6799920), *Lactobacillus animalis* (32012274), *Vibrio alginolyticus* (29869205), *Bacteroides thetaiotaomicron* (31617701), *Bacteroides thetaiotaomicron* (31617140), *Bacteroides cel-lulosilyticus* (29608790), *Bacteroides ovatus* (29453452),

Bacillus mycoides (29402181), *Chlamydomonas reinhardtii* (5726206), *Fusobacterium periodonticum* (35833538), *Selenomonas flueggei* (32477557), *Selenomonas noxia* (32475880), *Anaerococcus hydrogenalis* (32462628), *Centipeda periodontii* (32173931), *Centipeda periodontii* (32173899), *Streptococcus thermophilus* (31938326), *Enterococcus durans* (31916360), *Fusobacterium nucleatum* (31730399), *Anaerostipes hadrus* (31625694), *Anaerostipes hadrus* (31623667), *Enterococcus haemoperoxidus* (29838940), *Gardnerella vaginalis* (29692621), *Streptococcus salivarius* (29397526), *Klebsiella oxytoca* (29379245), *Bifidobacterium breve* (29241363), *Actinomyces odontolyticus* (25045153), *Haemophilus ducreyi* (24944624), *Archaeoglobus fulgidus* (24793671), *Streptococcus uberis* (24161511), *Fusobacterium nucleatum* subsp. *animalis* (23369066), *Corynebacterium accolens* (23249616), *Archaeoglobus veneficus* (10394332), *Prevotella melaninogenica* (9497682), *Aeromonas salmonicida* subsp. *salmonicida* (4997325), *Pyrobaculum islandicum* (4616932), *Thermofilum pendens* (4600420), *Bifidobacterium adolescentis* (4556560), *Listeria monocytogenes* (986485), *Bifidobacterium thermophilum* (35776852), *Methanothermobacter* sp. CaT2 (24854111), *Streptococcus pyogenes* (901706), *Exiguobacterium sibiricum* (31768748), *Clostridioides difficile* (4916015), *Clostridioides difficile* (4913022), *Vibrio parahaemolyticus* (1192264), *Yersinia enterocolitica* subsp. *enterocolitica* (4712948), *Enterococcus cecorum* (29475065), *Bifidobacterium pseudolongum* (34879480), *Methanothermus fervidus* (9962832), *Methanothermus fervidus* (9962056), *Corynebacterium simulans* (29536891), *Thermoproteus uzonensis* (10359872), *Vulcanisaeta distributa* (9752274), *Streptococcus mitis* (8799048), *Ferroglobus placidus* (8778420), *Streptococcus suis* (8153745), *Clostridium novyi* (4541619), *Streptococcus mutans* (1029528), *Thermosynechococcus elongatus* (1010568), *Chlorobium tepidum* (1007539), *Fusobacterium nucleatum* subsp. *nucleatum* (993139), *Streptococcus pneumoniae* (933787), *Clostridium barattii* (31579258), *Enterococcus mundtii* (31547246), *Prevotella ruminicola* (31500814), *Aeromonas hydrophila* subsp. *hydrophila* (4490168), *Aeromonas hydrophila* subsp. *hydrophila* (4487541), *Clostridium acetobutylicum* (1117604), *Chromobacterium subtsugae* (31604683), *Gilliamella apicola* (29849369), *Klebsiella pneumoniae* subsp. *pneumoniae* (11846825), *Enterobacter cloacae* subsp. *cloacae* (9125235), *Escherichia coli* (7150298), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (1252363), *Salmonella enterica* subsp. *enterica* serovar *Typhi* (1247322), *Bacillus cereus* (1202845), *Bacteroides thetaiotaomicron* (1074343), *Bacteroides thetaiotaomicron* (1071815), *Bacillus coagulans* (29814250), *Bacteroides cellulosilyticus* (29610027), *Bacillus anthracis* (2850719), *Monoraphidium neglectum* (25735215), *Monoraphidium neglectum* (25727595), *Alloocardovia omnicolens* (35868062), *Actinomyces neuii* subsp. *neuii* (35867196), *Acetanaeraerobium sticklandii* (35557713), *Exiguobacterium undae* (32084128), *Paenibacillus pabuli* (32034589), *Paenibacillus etheri* (32019864), *Actinomyces oris* (31655321), *Vibrio alginolyticus* (31651465), *Brochotricha thermosphacta* (29820407), *Lactobacillus sakei* subsp. *sakei* (29638315), *Anoxybacillus gonensis* (29574914), variants thereof as well as fragments thereof. In an embodiment, the PFLA protein is derived from the genus *Bifidobacterium* and in some embodiments from the species *Bifidobacterium adolescentis*. In an embodiment, the yeast host cell

expresses an heterologous PFLA polypeptide having the amino acid sequence of SEQ ID NO: 13, a variant thereof or a fragment thereof.

[0088] Embodiments of PFLB can be derived, without limitation, from the following (the number in brackets correspond to the Gene ID number): *Escherichia coli* (945514), *Shewanella oneidensis* (1170601), *Actinobacillus suis* (34292499), *Finegoldia magna* (34165044), *Streptococcus cristatus* (29901775), *Enterococcus hirae* (13176625), *Bacillus* (3031414), *Providencia alcalifaciens* (34345353), *Lactococcus garvieae* (34203444), *Butyrivibrio proteoclasticus* (31781354), *Teredinibacter turnerae* (29651613), *Chromobacterium violaceum* (24945652), *Vibrio campbellii* (5554880), *Vibrio campbellii* (5554796), *Rahnella aquatilis* HX2 (34351700), *Serratia rubidaea* (32375076), *Kosakonia sacchari* SP1 (23845740), *Shewanella baltica* (11772863), *Streptomyces acidiscabies* (33082309), *Streptomyces davaonensis* (31227068), *Parabacteroides distasonis* (5308541), *Bacteroides vulgatus* (5303841), *Fibrobacter succinogenes* subsp. *succinogenes* (34755392), *Photobacterium damselae* subsp. *Damselae* (34512678), *Enterococcus gilvus* (34361749), *Enterococcus gilvus* (34360863), *Enterococcus malodoratus* (34355213), *Enterococcus malodoratus* (34354022), *Akkermansia muciniphila* (34174913), *Lactobacillus curvatus* (33995135), *Dickeya zeae* (33924934), *Bacteroides oleciplenus* (32502326), *Micromonospora aurantiaca* (32162989), *Seletonomas ruminantium* subsp. *lactilytica* (31522408), *Fusobacterium necrophorum* subsp. *funduliforme* (31520832), *Bacteroides uniformis* (31507007), *Streptomyces rimosus* subsp. *Rimosus* (29531908), *Clostridium innocuum* (26150740), *Haemophilus*] *ducreyi* (24944556), *Clostridium bolteae* (23114829), *Vibrio tasmaniensis* (7160644), *Aeromonas salmonicida* subsp. *salmonicida* (4997718), *Listeria monocytogenes* (986171), *Enterococcus faecalis* (1200511), *Lactobacillus plantarum* (1064019), *Vibrio fischeri* (3278780), *Lactobacillus sakei* (33973511), *Gardnerella vaginalis* (9904192), *Vibrio vulnificus* (33954428), *Vibrio toranzoniae* (34373229), *Anaerostipes hadrus* (34240161), *Edwardsiella anguillarum* (33940299), *Edwardsiella anguillarum* (33937990), *Lonsdalea quercina* subsp. *Quercina* (33074710), *Enterococcus faecium* (12999834), *Aeromonas hydrophila* subsp. *hydrophila* (4489100), *Clostridium acetobutylicum* (1117163), *Escherichia coli* (7151395), *Shigella dysenteriae* (3795966), *Bacillus thuringiensis* serovar *konkukian* (2856201), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (1252491), *Shigella flexneri* (1023824), *Streptomyces griseoruber* (32320336), *Cryobacterium flavum* (35898977), *Ruminococcus gnavus* (35895748), *Bacillus acidiceler* (34874555), *Lactococcus piscium* (34864362), *Vibrio mediterranei* (34766270), *Faecalibacterium prausnitzii* (34753200), *Prevotella intermedia* (34516966), *Photobacterium damselae* subsp. *Damselae* (34509286), *Pseudobutyrivibrio ruminis* (34419894), *Melissococcus plutonius* (34408953), *Streptococcus galloyticus* subsp. *galloyticus* (34398704), *Enterobacter hormaechei* subsp. *Steigerwaltii* (34155981), *Enterobacter hormaechei* subsp. *Steigerwaltii* (34152298), *Streptomyces venezuelae* (34036549), *Shewanella algae* (34009243), *Lactobacillus agilis* (33976013), *Streptococcus equinus* (33961013), *Neisseria sicca* (33952517), *Kitasatospora purpeofusca* (32375782), *Paenibacillus borealis* (29549449), *Vibrio fluvialis* (29387150), *Aliivibrio wodanis* (28542465), *Aliivibrio wodanis* (28541256), *Escherichia*

coli (7157421), *Salmonella enterica* subsp. *enterica* serovar *Typhi* (1247405), *Yersinia pestis* (1174224), *Yersinia enterocolitica* subsp. *enterocolitica* (4713334), *Streptococcus suis* (8155093), *Escherichia coli* (947854), *Escherichia coli* (946315), *Escherichia coli* (945513), *Escherichia coli* (948904), *Escherichia coli* (917731), *Yersinia enterocolitica* subsp. *enterocolitica* (4714349), variants thereof as well as fragments thereof. In an embodiment, the PFLB protein is derived from the genus *Bifidobacterium* and in some embodiments from the species *Bifidobacterium adolescentis*. In such embodiments, the PFLB protein can have the amino acid sequence of SEQ ID NO: 7, be a variant of SEQ ID NO: 7 or be a fragment of SEQ ID NO: 7. In another embodiment, the recombinant yeast host cell comprises a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 16 or 17. In an embodiment, the heterologous nucleic acid molecule encoding the PFLB protein is present in at least one, two, three, four, five or more copies in the recombinant yeast host cell. In still another embodiment, the heterologous nucleic acid molecule encoding the PFLB protein is present in no more than five, four, three, two or one copy/ies in the recombinant yeast host cell. The yeast host cell can be modified to express an heterologous PFLB polypeptide having the amino acid sequence of SEQ ID NO: 14, a variant thereof or a fragment thereof.

[0089] In some embodiments, the recombinant yeast host cell comprises a second genetic modification for expressing a PFLA protein, a PFLB protein or a combination. In a specific embodiment, the recombinant yeast host cell comprises a second genetic modification for expressing a PFLA protein and a PFLB protein which can, in some embodiments, be provided on distinct heterologous nucleic acid molecules. As indicated below, the recombinant yeast host cell can also include additional genetic modifications to provide or increase its ability to transform acetyl-CoA into an alcohol such as ethanol.

[0090] Alternatively or in combination, the yeast host cell can bear one or more genetic modifications for utilizing acetyl-CoA for example, by providing or increasing acetaldehyde and/or alcohol dehydrogenase activity. Acetyl-CoA can be converted to an alcohol such as ethanol using second an acetaldehyde dehydrogenase and then an alcohol dehydrogenase. Acylating acetaldehyde dehydrogenases (E.C. 1.2.1.10) are known to catalyze the conversion of acetaldehyde into acetyl-CoA in the presence of CoA. Alcohol dehydrogenases (E.C. 1.1.1.1) are known to be able to catalyze the conversion of acetaldehyde into ethanol. The acetaldehyde dehydrogenase and alcohol dehydrogenase activity can be provided by a single protein (e.g., a bifunctional acetaldehyde/alcohol dehydrogenase) or by a combination of more than one protein (e.g., an acetaldehyde dehydrogenase and an alcohol dehydrogenase). In embodiments in which the acetaldehyde/alcohol dehydrogenase activity is provided by more than one protein, it may not be necessary to provide the combination of proteins in a recombinant form in the recombinant yeast host cell as the cell may have some pre-existing acetaldehyde or alcohol dehydrogenase activity. In such embodiments, the sixth genetic modification can include providing one or more heterologous nucleic acid molecule encoding one or more of an heterologous acetaldehyde dehydrogenase (AADH), an heterologous alcohol dehydrogenase (ADH) and/or heterologous bifunctional acetaldehyde/alcohol dehydrogenases (ADHE). For example, the sixth genetic modification

can comprise introducing an heterologous nucleic acid molecule encoding an acetaldehyde dehydrogenase. In another example, the sixth genetic modification can comprise introducing an heterologous nucleic acid molecule encoding an alcohol dehydrogenase. In still another example, the sixth genetic modification can comprise introducing at least two heterologous nucleic acid molecules, a second one encoding an heterologous acetaldehyde dehydrogenase and a second one encoding an heterologous alcohol dehydrogenase. In another embodiment, the sixth genetic modification comprises introducing an heterologous nucleic acid encoding an heterologous bifunctional acetaldehyde/alcohol dehydrogenases (AADH) such as those described in U.S. Pat. No. 8,956,851 and WO 2015/023989. Heterologous AADHs of the present disclosure include, but are not limited to, the ADHE polypeptides or a polypeptide encoded by an adhe gene ortholog. In an embodiment, the AADH has the amino acid sequence of SEQ ID NO: 15, is a variant of the amino acid sequence of SEQ ID NO: 15 or is a fragment of the amino acid sequence of SEQ ID NO: 15. In such embodiment, the genetic modification can comprise introducing an heterologous nucleic acid molecule encoding a protein having the amino acid sequence of SEQ ID NO: 15, being a variant of the amino acid sequence of SEQ ID NO: 15 or being a fragment of the amino acid sequence of SEQ ID NO: 15.

[0091] The yeast host cell described herein can be provided as a combination with the bacterial host cell described herein. In such combination, the yeast host cell can be provided in a distinct container from the bacterial host cell. The yeast host cell can be provided as a cell concentrate. The cell concentrate comprising the yeast host cell can be obtained, for example, by propagating the yeast host cells in a culture medium and removing at least one components of the medium comprising the propagated yeast host cell. This can be done, for example, by dehydrating, filtering (including ultra-filtrating) and/or centrifuging the medium comprising the propagated yeast host cell. In an embodiment, the yeast host cell is provided as a cream in the combination.

Bacterial Host Cell

[0092] In the context of the present disclosure, the host cell is a bacterium and, in some embodiments, a lactic acid bacterium (LAB). As it is known in the art, LAB are a group of Gram-positive bacteria, non-respiring non-spore-forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. Bacterial genus of LAB include, but are not limited to, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Bacterial species of LAB include, but are not limited to, *Lactococcus lactis*, *Lactococcus garviae*, *Lactococcus raffinolactis*, *Lactococcus plantarum*, *Oenococcus oeni*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Carnococcus allantoicus*, *Carnobacterium gallinarum*, *Vagococcus fessus*, *Streptococcus thermophilus*, *Enterococcus phoeniculicola*, *Enterococcus plantarum*, *Enterococcus raffinosus*, *Enterococcus avium*, *Enterococcus pallens*, *Enterococcus hermanniensis*, *Enterococcus faecalis*, and *Enterococcus faecium*. In an embodiment, the LAB is a *Lactobacillus* and, in some additional embodiment, the *Lactobacillus* species is *L. acetotolerans*, *L. acidifarinae*, *L. acidipiscis*, *L. acidophilus*, *L. agilis*, *L. algidus*, *L. alimen-*

*tarius, L. amylolyticus, L. amylophilus, L. amylotrophicus, L. amylovorus, L. animalis, L. antri, L. apodemi, L. aviarius, L. bifermentans, L. brevis, L. buchneri, L. camelliae, L. casei, L. catenaformis, L. ceti, L. coleohominis, L. collinoides, L. composti, L. concavus, L. coryniformis, L. crispatus, L. crustorum, L. curvatus, L. delbrueckii (including L. delbrueckii subsp. *bulgaricus*, L. delbrueckii subsp. *delbrueckii*, L. delbrueckii subsp. *lactis*), L. dextrinicus, L. diolivorans, L. equi, L. equigenerosi, L. farraginis, L. sarciniminis, L. fermentum, L. fornicalis, L. fructivorans, L. frumenti, L. fuchuensis, L. gallinarum, L. gasseri, L. gastricus, L. ghanensis, L. graminis, L. ammesii, L. hamsteri, L. harbinensis, L. hayakitensis, L. helveticus, L. hilgardii, L. omohiochii, L. iners, L. ingluviei, L. intestinalis, L. jensenii, L. johnsonii, L. kalixensis, L. efranofaciens, L. kefiri, L. kimchii, L. kitasatonis, L. kunkeei, L. leichmannii, L. lindneri, L. alefermentans, L. mali, L. manihotivorans, L. mindensis, L. mucosae, L. murinus, L. nagelii, L. namurensis, L. nantensis, L. oligofermentans, L. oris, L. panis, L. pantheris, L. parabrevis, L. parabuchneri, L. paracasei, L. paracollinoides, L. parafarraginis, L. parakefiri, L. aralimentarius, L. parapantarum, L. pentosus, L. perolens, L. plantarum, L. pontis, L. protectus, L. psittaci, L. rennini, L. reuteri, L. rhamnosus, L. rimae, L. rogosae, L. rossiae, L. ruminis, L. saerimneri, L. sakei, L. salivarius, L. sanfranciscensis, L. satsumensis, L. secaliphilus, L. sharpeae, L. siliquinis, L. spicheri, L. suebicus, L. thailandensis, L. ultunensis, L. vaccinostercus, L. vaginalis, L. versmoldensis, L. vini, L. vitulinus, L. zae or L. zymae.* In some embodiments, the bacterial host cell is *L. paracasei* and in some embodiments, *L. paracasei* 12A. For example, the bacterial host cell can be one of those described in WO 2018/013791.

[0093] The bacterial host cell of the present disclosure can have a second metabolic pathway comprising one or more second enzymes for producing a second metabolic product (from the first metabolic product). The bacterial host cell can have native enzymes present in the second metabolic pathway and be capable to produce the second metabolic product. Alternatively or in combination, the bacterial host cell can include one or more genetic modification to increase the activity of the one or more enzymes in the second metabolic pathway. The increased in activity is due at least in part to the introduction of one or more second genetic modifications in a native bacterial host cell to obtain the bacterial host cell. As such, the activity of the one or more second enzymes of the bacterial host cell is considered “increased” because it is higher than the activity of the one or more second enzymes in the native bacterial host cell (e.g., prior to the introduction of the one or more second genetic modifications). The one or more second genetic modifications is not limited to a specific modification provided that it does increase the activity, and in some embodiments, the expression of the one or more second enzymes. For example, the one or more second genetic modifications can include the addition of a promoter to increase the expression of the one or more (endogenous) second enzymes. Alternatively or in addition, the one or more second genetic modifications can include the introduction of one or more copies of a gene(s) encoding the one or more second (heterologous) enzymes in the bacterial host cell.

[0094] In the embodiment in which the first metabolic product is a carbohydrate such as trehalose, the second metabolic product can be ethanol and involve the anabolism of glucose-6-phosphate. In such embodiment, the bacterial

host cell can have native activity in a PTS transporter, a trehalose-6-phosphate, an hexokinase and/or be genetically modified to provide or increase biological activity in at least one of a PTS transporter, a trehalose-6-phosphate or an hexokinase. In another embodiment in which the first metabolic product is a carbohydrate such as trehalose, the second metabolic product can be ethanol and involve the anabolism of acetaldehyde. In such embodiment, the bacterial host cell can have native pyruvate decarboxylase activity and/or be genetically modified to provide or increase pyruvate decarboxylase activity. In still another embodiment in which the first metabolic product is a carbohydrate such as trehalose, the second metabolic product can be ethanol. In such embodiment, the bacterial host cell can have native alcohol dehydrogenase activity and/or be genetically modified to provide or increase alcohol dehydrogenase activity. In an embodiment, the bacterial host cell has increased biological activity of a pyruvate decarboxylase, but not of the alcohol dehydrogenase. In another embodiment, the bacterial host cell has increased biological activity of an alcohol dehydrogenase, but not of the pyruvate decarboxylase. In still another embodiment, the bacterial host cell has increased biological activity in both a pyruvate decarboxylase and an alcohol dehydrogenase. As indicated above, this can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous pyruvate decarboxylase and/or the endogenous alcohol dehydrogenase. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding an heterologous a pyruvate decarboxylase and/or an heterologous alcohol dehydrogenase.

[0095] In another embodiment in which the first metabolic product is an organic acid (or its associated ester), such as acetic acid (or acetate), the second metabolic product can be ethanol and involve the anabolism of the acetic acid (or acetate). As used in the context of the present disclosure, the expression “organic acid” includes associated organic esters which can be hydrolyzed into the organic acid. In such embodiment, the bacterial host cell have native citrate lyase activity (to convert citric acid/citrate into acetic acid/acetate and oxaloacetate) and/or be genetically modified to provide or increase citrate lyase activity. Optionally, the bacterial host cell can have native pyruvate decarboxylase activity and/or be genetically modified to provide or increase pyruvate decarboxylase activity. Alternatively or in combination, the bacterial host cell can have native alcohol dehydrogenase activity and/or be genetically modified to provide or increase alcohol dehydrogenase activity. Alternatively or in combination, the bacterial host cell can have a native oxaloacetate decarboxylase and/or be genetically modified to provide or increase oxaloacetate decarboxylase activity. As indicated above, this can be done by introducing a strong and/or constitutive promoter to increase the expression of the endogenous citrate lyase, the endogenous pyruvate decarboxylase, the endogenous alcohol dehydrogenase and/or the endogenous oxaloacetate decarboxylase. Alternatively or in combination, this can also be done by introducing at least one copy of one or more heterologous nucleic acid molecules encoding an heterologous citrate lyse, an heterologous a pyruvate decarboxylase, an heterologous alcohol dehydrogenase and/or an heterologous oxaloacetate decarboxylase.

[0096] As used herein, the term “citrate lyase” refers to an enzyme catalyzing the conversion of citrate into acetate and oxaloacetate (EC 4.1.3.6). In some embodiments, the citrate lyase is obtained from a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the citrate lyase can have the amino acid sequence of SEQ ID NO: 17, be a variant of the amino acid sequence of SEQ ID NO: 17 or be a fragment of the amino acid of SEQ ID NO: 17 or a variant thereof. Still in additional embodiments, the citrate lyase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 18, a variant of the nucleic acid sequence of SEQ ID NO: 18 or a fragment of the nucleic acid sequence of SEQ ID NO: 18 or variant thereof. In some embodiments, the citrate lyase can comprise the beta chain of the citrate lyase of a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the beta chain of the citrate lyase can have the amino acid sequence of SEQ ID NO: 19, be a variant of the amino acid sequence of SEQ ID NO: 19 or be a fragment of the amino acid of SEQ ID NO: 19 or a variant thereof. Still in additional embodiments, the beta chain of the citrate lyase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 20, a variant of the nucleic acid sequence of SEQ ID NO: 20 or a fragment of the nucleic acid sequence of SEQ ID NO: 20 or variant thereof. In some embodiments, the citrate lyase can comprise the gamma chain of the citrate lyase of a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the gamma chain of the citrate lyase can have the amino acid sequence of SEQ ID NO: 21, be a variant of the amino acid sequence of SEQ ID NO: 21 or be a fragment of the amino acid of SEQ ID NO: 21 or a variant thereof. Still in additional embodiments, the gamma chain of the citrate lyase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 22, a variant of the nucleic acid sequence of SEQ ID NO: 22 or a fragment of the nucleic acid sequence of SEQ ID NO: 22 or variant thereof.

[0097] As used herein, the term “oxaloacetate decarboxylase” refers to an enzyme catalyzing the decarboxylation of oxaloacetate to pyruvate and carbon dioxide (E.C. 4.1.1.3). In some embodiments, the oxaloacetate decarboxylase is obtained from a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the oxaloacetate decarboxylase can have an alpha chain comprising the amino acid sequence of SEQ ID NO: 23, be a variant of the amino acid sequence of SEQ ID NO: 23 or be a fragment of the amino acid of SEQ ID NO: 23 or a variant thereof. Still in additional embodiments, the alpha chain of the oxaloacetate decarboxylase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 24, a variant of the nucleic acid sequence of SEQ ID NO: 24 or a fragment of the nucleic acid sequence of SEQ ID NO: 24 or variant thereof. In some embodiments, the oxaloacetate decarboxylase can comprise a beta chain of obtained from a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the beta chain of the oxaloacetate decarboxylase can have the amino acid sequence of SEQ ID NO: 25, be a variant of the amino acid sequence of SEQ ID NO: 25 or be a fragment of the amino acid of SEQ ID NO: 25 or a variant thereof. Still in additional embodiments, the beta chain of the oxaloacetate decarboxylase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 1 to 3.

NO: 26, a variant of the nucleic acid sequence of SEQ ID NO: 26 or a fragment of the nucleic acid sequence of SEQ ID NO: 26 or variant thereof. In some embodiments, the oxaloacetate decarboxylase can comprise a gamma chain of obtained from a *Lactobacillus* sp., such as for example, a *Lactobacillus paracasei*. In such embodiment, the gamma chain of the oxaloacetate decarboxylase can have the amino acid sequence of SEQ ID NO: 55, be a variant of the amino acid sequence of SEQ ID NO: 55 or be a fragment of the amino acid of SEQ ID NO: 55 or a variant thereof. Still in additional embodiments, the gamma chain of the oxaloacetate decarboxylase can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 56, a variant of the nucleic acid sequence of SEQ ID NO: 56 or a fragment of the nucleic acid sequence of SEQ ID NO: 56 or variant thereof. In some additional embodiments, the oxaloacetate decarboxylase is a trimeric polypeptide comprises at least one of an alpha chain (having the amino acid sequence of SEQ ID NO: 23, a variant thereof or a fragment thereof), a beta chain (having the amino acid sequence of SEQ ID NO: 25, a variant thereof or a fragment thereof) or a gamma chain (having the amino acid sequence of SEQ ID NO: 55, a variant thereof or a fragment thereof). In some additional embodiments, the oxaloacetate decarboxylase is a trimeric polypeptide comprises at least two of an alpha chain (having the amino acid sequence of SEQ ID NO: 23, a variant thereof or a fragment thereof), a beta chain (having the amino acid sequence of SEQ ID NO: 25, a variant thereof or a fragment thereof) or a gamma chain (having the amino acid sequence of SEQ ID NO: 55, a variant thereof or a fragment thereof). In some additional embodiments, the oxaloacetate decarboxylase is a trimeric polypeptide comprises an alpha chain (having the amino acid sequence of SEQ ID NO: 23, a variant thereof or a fragment thereof), a beta chain (having the amino acid sequence of SEQ ID NO: 25, a variant thereof or a fragment thereof) and a gamma chain (having the amino acid sequence of SEQ ID NO: 55, a variant thereof or a fragment thereof).

[0098] As used herein, the term “pyruvate decarboxylase” refers to an enzyme catalyzing the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. In *Zymonas mobilis*, the pyruvate decarboxylase gene is referred to as PDC (Gene ID: 33073732) and could be used in the bacterial host cell of the present disclosure. In some additional embodiments, the pyruvate decarboxylase polypeptide can be from *Lactobacillus florum* (Accession Number WP_009166425.1), *Lactobacillus fructivorans* (Accession Number WP_039145143.1), *Lactobacillus lindneri* (Accession Number WP_065866149.1), *Lactococcus lactis* (Accession Number WP_104141789.1), *Carnobacterium gallinarum* (Accession Number WP_034563038.1), *Enterococcus plantarum* (Accession Number WP_069654378.1), *Clostridium acetobutylicum* (Accession Number NP_149189.1), *Bacillus megaterium* (Accession Number WP_075420723.1) or *Bacillus thuringiensis* (Accession Number WP_052587756.1). In the bacterial host cell of the present disclosure, the pyruvate decarboxylase can have the amino acid of SEQ ID NO: 4, be a variant of SEQ ID NO: 4 or a fragment of SEQ ID NO: 4. In some specific embodiments, the bacterial host cell of the present disclosure can express an heterologous nucleic acid molecule comprising the nucleic acid sequence of any one of SEQ ID NO: 1 to 3.

[0099] As used herein, the term “alcohol dehydrogenase” refers to an enzyme of the EC 1.1.1.1 class. In some embodiments, the alcohol dehydrogenase is an iron-containing alcohol dehydrogenase. The alcohol dehydrogenase that can be expressed in the bacterial host cell includes, but is not limited to, ADH4 from *Saccharomyces cerevisiae*, ADHB from *Zymonas mobilis*, FUCO from *Escherichia coli*, ADHE from *Escherichia coli*, ADH1 from *Clostridium acetobutylicum*, ADH1 from *Entamoeba nuttalli*, BDHA from *Clostridium acetobutylicum*, BDHB from *Clostridium acetobutylicum*, 4HBD from *Clostridium kluyveri*, DHAT from *Citrobacter freundii* or DHAT from *Klebsiella pneumoniae*. In an embodiment, the alcohol dehydrogenase can be ADHB from *Zymonas mobilis* (Gene ID: AHJ71151.1), *Lactobacillus reuteri* (Accession Number: KRK51011.1), *Lactobacillus mucosae* (Accession Number WP_048345394.1), *Lactobacillus brevis* (Accession Number WP_003553163.1) or *Streptococcus thermophiles* (Accession Number WP_113870363.1). In the bacterial host cell of the present disclosure, the pyruvate decarboxylase can have the amino acid of SEQ ID NO: 8, be a variant of SEQ ID NO: 8 or a fragment of SEQ ID NO: 8. In some specific embodiments, the bacterial host cell of the present disclosure can express an heterologous nucleic acid molecule comprising the nucleic acid sequence of any one of SEQ ID NO: 5 to 7.

[0100] In a specific embodiment, the recombinant yeast host cell can express an heterologous polypeptide having NADPH-dependent alcohol dehydrogenase activity. The protein having NADPH-dependent alcohol dehydrogenase activity can be an ADH polypeptide (for example from *Entamoeba* sp., including *Entamoeba nuttalli* (such as, for example, the one having the amino acid sequence of SEQ ID NO: 45), an ADH1 polypeptide variant, an ADH1 polypeptide fragment or a polypeptide encoded by an ADH1 gene ortholog/paralog. In some specific embodiments, the bacterial host cell of the present disclosure can express an heterologous nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 46. In yet another embodiment, the heterologous gene coding for the NADPH-dependent alcohol dehydrogenase protein is present in one, two, three, four or more copies in the recombinant microbial host cell. In the embodiments in which the first metabolic product is a sugar alcohol such as mannitol, the second metabolic product can be ethanol and involve the anabolism of fructose-6-phosphate.

[0101] In such embodiment, the bacterial host cell can be selected for its ability to utilize mannitol because it comprises a native mannitol utilization operon. In such embodiment, it is possible to use the bacterial host cell without introducing a genetic modification to allow mannitol utilization. Alternatively or in combination, the bacterial host cell can have increased biological activity in one or more proteins encoded by the genes of the mannitol utilization operon. For example, the bacterial host cell can have increase biological activity in a mannitol-1-phosphatase 5-dehydrogenase (such as MTLD2) and/or a mannitol transporter. In an embodiment, the MTLD2 polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLD2 polypeptide can have the amino acid sequence of SEQ ID NO: 39, be a variant of the amino acid sequence of SEQ ID NO: 39 or be a fragment of the amino acid sequence of SEQ ID NO: 39 or a variant thereof. In some additional embodiments, the

MTLD2 polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 40, a variant of the nucleic acid sequence of SEQ ID NO: 40 or a fragment of the nucleic acid sequence of SEQ ID NO: 40 or a fragment thereof. In an embodiment, the MTLCB polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLCB polypeptide can have the amino acid sequence of SEQ ID NO: 41, be a variant of the amino acid sequence of SEQ ID NO: 41 or be a fragment of the amino acid sequence of SEQ ID NO: 41 or a variant thereof. In some additional embodiments, the MTLCB polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 42, a variant of the nucleic acid sequence of SEQ ID NO: 42 or a fragment of the nucleic acid sequence of SEQ ID NO: 42 or a fragment thereof. In an embodiment, the MTLA polypeptide can be from *Lactobacillus* sp., such as, for example *Lactobacillus casei*. In some embodiments, the MTLA polypeptide can have the amino acid sequence of SEQ ID NO: 43, be a variant of the amino acid sequence of SEQ ID NO: 43 or be a fragment of the amino acid sequence of SEQ ID NO: 43 or a variant thereof. In some additional embodiments, the MTLA polypeptide can be encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 44, a variant of the nucleic acid sequence of SEQ ID NO: 44 or a fragment of the nucleic acid sequence of SEQ ID NO: 44 or a fragment thereof.

[0102] In the embodiments in which the first metabolic product is a sugar alcohol such as sorbitol, the second metabolic product can be ethanol and involve the anabolism of fructose-6-phosphate. In such embodiment, the bacterial host cell can be selected for its ability to utilize sorbitol because it comprises a native sorbitol utilization operon. In such embodiment, it is possible to use the bacterial host cell without introducing a genetic modification to allow sorbitol utilization. Alternatively or in combination, the bacterial host cell can have increased biological activity in one or more protein encoded by the genes of the sorbitol utilization operon. For example, the bacterial host cell can have increase biological activity in one or more proteins of the sorbitol operon which includes the gutF (encoding a sorbitol-6-phosphate dehydrogenase or the GUTF polypeptide), gutC (encoding the transporter subunit C or the GUTC polypeptide), gutB (encoding the transporter subunit B or the GUTB polypeptide) and gutA (encoding the transporter subunit A or the GUTA polypeptide) genes. In an embodiment, the GUTF polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTF polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 31, be a variant of the amino acid sequence of SEQ ID NO: 31 or be a fragment of the amino acid sequence of SEQ ID NO: 31 or a variant thereof. In an embodiment, the GUTF polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 32, being a variant of the nucleic acid sequence of SEQ ID NO: 32 or being a fragment of the nucleic acid sequence or SEQ ID NO: 32 or a variant thereof. In an embodiment, the GUTC polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTC polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 33, be a variant of the amino acid sequence of SEQ ID NO: 33 or be a fragment of the amino acid sequence of SEQ ID

NO: 33 or a variant thereof. In an embodiment, the GUTC polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 34, being a variant of the nucleic acid sequence of SEQ ID NO: 34 or being a fragment of the nucleic acid sequence or SEQ ID NO: 34 or a variant thereof. In an embodiment, the GUTB polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTB polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 35, be a variant of the amino acid sequence of SEQ ID NO: 35 or be a fragment of the amino acid sequence of SEQ ID NO: 35 or a variant thereof. In an embodiment, the GUTB polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 36, being a variant of the nucleic acid sequence of SEQ ID NO: 36 or being a fragment of the nucleic acid sequence or SEQ ID NO: 36 or a variant thereof. In an embodiment, the GUTA polypeptide is from *Lactobacillus* sp., such as, for example *Lactobacillus paracasei*. In such embodiment, the GUTA polypeptide can have, for example, the amino acid sequence of SEQ ID NO: 37, be a variant of the amino acid sequence of SEQ ID NO: 37 or be a fragment of the amino acid sequence of SEQ ID NO: 37 or a variant thereof. In an embodiment, the GUTA polypeptide is encoded by an heterologous nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 38, being a variant of the nucleic acid sequence of SEQ ID NO: 38 or being a fragment of the nucleic acid sequence or SEQ ID NO: 38 or a variant thereof.

[0103] In the embodiments in which the first metabolic product is a sugar alcohol such as glycerol, the second metabolic product can be ethanol and involved the anabolism of dihydroxyacetone-phosphate. The bacterial host cell can have native or engineered activity in a second metabolic pathway, e.g., the glycerol dehydrogenase/DHA kinase pathway. In such embodiment, the bacterial host cell comprises native or engineered increased biological activity in one or more of a glycerol hydrogenase and/or dihydroxyacetone kinase. Alternatively or in combination, the bacterial host cell can have native or engineered activity in another second metabolic pathway, e.g., the glycerol kinase/glycerol-3-phosphate dehydrogenase pathway. In such embodiment, the bacterial host cell comprises native or engineered increased biological activity in one or more of a glycerol kinase and/or a glycerol-3-phosphate dehydrogenase. Alternatively or in combination, the bacterial host cell can have a native and/or be genetically modified to provide or increase a glycerol facilitator activity.

[0104] In some embodiments, the bacterial host cell can be further modified to inactivate one or more endogenous genes. In the context of the present disclosure, the inactivation of a gene refers to the removal of at least one nucleic acid residue so as to impede the expression of the endogenous genes. The at least one nucleic acid residue can be removed in the coding or the non-coding region of the gene. In some embodiments, the entire coding region of a gene is removed to inactivate the gene. In some additional embodiments, one or more additional nucleic acid residues can be added at the location at which the deletion occurred.

[0105] In a specific embodiment, especially when the trehalose or acetic acid/acetate is the first metabolic product, the bacterial host cell can be modified to as to decrease is lactate dehydrogenase activity. As used in the context of the present disclosure, the expression "lactate dehydrogenase"

refer to an enzyme of the E.C. 1.1.1.27 class which is capable of catalyzing the conversion of pyruvic acid into lactate. The bacterial host cells can thus have one or more gene coding for a protein having lactate dehydrogenase activity which is inactivated (via partial or total deletion of the gene). In bacteria, the ldh1, ldh2, ldh3 and ldh4 genes encode proteins having lactate dehydrogenase activity. Some bacteria may contain as many as six or more such genes (i.e., ldh5, ldh6, etc.) In an embodiment, at least one of the ldh1, ldh2, ldh3 and ldh4 genes, their corresponding orthologs and paralogs is inactivated in the bacterial host cell. In an embodiment, only one of the ldh genes is inactivated in the bacterial host cell. For example, in the bacterial host cell of the present disclosure, only the ldh1 gene can be inactivated. In another embodiment, at least two of the ldh genes are inactivated in the bacterial host cell. In another embodiment, only two of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, at least three of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, only three of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, at least four of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, only four of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, at least five of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, only five of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, at least six of the ldh genes are inactivated in the bacterial host cell. In a further embodiment, only six of the ldh genes are inactivated in the bacterial host cell. In still another embodiment, all of the ldh genes are inactivated in the bacterial host cell.

[0106] In a specific embodiment, especially when trehalose or acetic acid/acetate is the first metabolic product, the bacterial host cell can be modified so as to decrease itsmannitol-1-phosphate 5-dehydrogenase activity. As used in the context of the present disclosure, the expression "mannitol-1-P 5-dehydrogenase" refer to an enzyme of the E.C. 1.1.1.17 class which is capable of catalyzing the conversion of mannitol into fructose-6-phosphate. The bacterial host cells can thus have one or more gene coding for a protein having mannitol dehydrogenase activity which is inactivated (via partial or total deletion of the gene). In bacteria, the mltd1 and mltd2 genes encode proteins havingmannitol-1-P 5-dehydrogenase activity. In an embodiment, at least one of the mltd1 and mltd2 genes, their corresponding orthologs and paralogs is inactivated in the bacterial host cell. In an embodiment, only one of the mltd1 and mltd2 genes is inactivated in the bacterial host cell. In another embodiment, both of the mltd1 and mltd2 genes are inactivated in the bacterial host cell.

[0107] The bacterial host cell described herein can be provided as a combination with the yeast cell described herein. In such combination, the bacterial host cell can be provided in a distinct container from the yeast cell. The bacterial host cell can be provided as a cell concentrate. The cell concentrate comprising the bacterial host cell can be obtained, for example, by propagating the bacterial host cells in a culture medium and removing at least one components of the medium comprising the propagated bacterial host cell. This can be done, for example, by dehydrating, filtering (including ultra-filtrating) and/or centrifuging the medium comprising the propagated bacterial host cell. In an

embodiment, the bacterial host cell is provided as a frozen concentrate in the combination.

Process of Using the Yeast Host Cell and the Bacterial Host Cell

[0108] The combination of the host cells described herein can be used to improve alcohol (e.g., ethanol) yield in a fermentation. As shown herein, some embodiments the combination of the yeast host cells and of the bacterial host cells are advantageous as they improve the robustness of the yeast host cells in the presence of a stressor during fermentation. The stressor can be, for example, a bacterial contamination, an increase in pH, a reduction in aeration, elevated temperatures, osmotic pressure or combinations thereof. In some embodiments, the process described herein can also be used to limit glucose and/or glycerol concentration during fermentation. In some other embodiments, the process described herein can also be used to limit or prevent contamination of the fermentation by other non-fermenting microorganisms (especially when the bacterial yeast host cell is capable of producing one or more bacteriocin).

[0109] The biomass that can be fermented with the combination of host cells described herein includes any type of biomass known in the art and described herein. For example, the biomass can include, but is not limited to, starch, sugar and lignocellulosic materials. Starch materials can include, but are not limited to, mashes such as corn, wheat, rye, barley, rice, or milo. Sugar materials can include, but are not limited to, sugar beets, artichoke tubers, sweet sorghum, molasses or cane. The terms "lignocellulosic material", "lignocellulosic substrate" and "cellulosic biomass" mean any type of biomass comprising cellulose, hemicellulose, lignin, or combinations thereof, such as but not limited to woody biomass, forage grasses, herbaceous energy crops, non-woody-plant biomass, agricultural wastes and/or agricultural residues, forestry residues and/or forestry wastes, paper-production sludge and/or waste paper sludge, wastewater-treatment sludge, municipal solid waste, corn fiber from wet and dry mill corn ethanol plants and sugar-processing residues. The terms "hemicellulosics", "hemicellulosic portions" and "hemicellulosic fractions" mean the non-lignin, non-cellulose elements of lignocellulosic material, such as but not limited to hemicellulose (i.e., comprising xyloglucan, xylan, glucuronoxylan, arabinoxylan, mannan, glucomannan and galactoglucomannan), pectins (e.g., homogalacturonans, rhamnogalacturonan I and II, and xylogalacturonan) and proteoglycans (e.g., arabinogalactan-protein, extensin, and proline-rich proteins). In some embodiments, the biomass can include and/or be supplemented with citric acid (especially when acetic acid or acetate is the first metabolic product).

[0110] In a non-limiting example, the lignocellulosic material can include, but is not limited to, woody biomass, such as recycled wood pulp fiber, sawdust, hardwood, softwood, and combinations thereof; grasses, such as switch grass, cord grass, rye grass, reed canary grass, miscanthus, or a combination thereof; sugar-processing residues, such as but not limited to sugar cane bagasse; agricultural wastes, such as but not limited to rice straw, rice hulls, barley straw, corn cobs, cereal straw, wheat straw, canola straw, oat straw, oat hulls, and corn fiber; stover, such as but not limited to soybean stover, corn stover; succulents, such as but not limited to, agave; and forestry wastes, such as but not limited to, recycled wood pulp fiber, sawdust, hardwood (e.g.,

poplar, oak, maple, birch, willow), softwood, or any combination thereof. Lignocellulosic material may comprise one species of fiber; alternatively, lignocellulosic material may comprise a mixture of fibers that originate from different lignocellulosic materials. Other lignocellulosic materials are agricultural wastes, such as cereal straws, including wheat straw, barley straw, canola straw and oat straw; corn fiber; stovers, such as corn stover and soybean stover; grasses, such as switch grass, reed canary grass, cord grass, and miscanthus; or combinations thereof.

[0111] Substrates for cellulose activity assays can be divided into two categories, soluble and insoluble, based on their solubility in water. Soluble substrates include celldextrins or derivatives, carboxymethyl cellulose (CMC), or hydroxyethyl cellulose (HEC). Insoluble substrates include crystalline cellulose, microcrystalline cellulose (Avicel), amorphous cellulose, such as phosphoric acid swollen cellulose (PASC), dyed or fluorescent cellulose, and pretreated lignocellulosic biomass. These substrates are generally highly ordered cellulosic material and thus only sparingly soluble.

[0112] It will be appreciated that suitable lignocellulosic material may be any feedstock that contains soluble and/or insoluble cellulose, where the insoluble cellulose may be in a crystalline or non-crystalline form. In various embodiments, the lignocellulosic biomass comprises, for example, wood, corn, corn stover, sawdust, bark, molasses, sugarcane, leaves, agricultural and forestry residues, grasses such as switchgrass, ruminant digestion products, municipal wastes, paper mill effluent, newspaper, cardboard or combinations thereof.

[0113] Paper sludge is also a viable feedstock for lactate or acetate production. Paper sludge is solid residue arising from pulping and paper-making, and is typically removed from process wastewater in a primary clarifier. The cost of disposing of wet sludge is a significant incentive to convert the material for other uses, such as conversion to ethanol. Processes provided by the present invention are widely applicable. Moreover, the saccharification and/or fermentation products may be used to produce ethanol or higher value added chemicals, such as organic acids, aromatics, esters, acetone and polymer intermediates.

[0114] The process of the present disclosure contacting the host cells described herein with a biomass so as to allow the conversion of at least a part of the biomass into the fermentation product. The fermented product can be an alcohol, such as, for example, ethanol, isopropanol, n-propanol, 1-butanol, methanol, acetone and/or 1, 2 propanediol. In an embodiment, the biomass or substrate to be hydrolyzed is a lignocellulosic biomass and, in some embodiments, it comprises starch (in a gelatinized or raw form). In the process of the present disclosure, the yeast host cells can be second contacted with the biomass. Alternatively, the bacterial host cells can be second contacted with the biomass. Also, in some embodiments, both the yeast host cells and the bacterial host cells can be contacted simultaneously with the biomass.

[0115] The fermentation process can be performed at temperatures of at least about 25° C., about 28° C., about 30° C., about 31° C., about 32° C., about 33° C., about 34° C., about 35° C., about 36° C., about 37° C., about 38° C., about 39° C., about 40° C., about 41° C., about 42° C., or about 50° C. In some embodiments, the process can be conducted at temperatures above about 30° C., about 31° C., about 32° C.,

about 33° C., about 34° C., about 35° C., about 36° C., about 37° C., about 38° C., about 39° C., about 40° C., about 41° C., about 42° C., or about 50° C.

[0116] In some embodiments, the process can be used to produce ethanol at a particular rate. For example, in some embodiments, ethanol is produced at a rate of at least about 0.1 mg per hour per liter, at least about 0.25 mg per hour per liter, at least about 0.5 mg per hour per liter, at least about 0.75 mg per hour per liter, at least about 1.0 mg per hour per liter, at least about 2.0 mg per hour per liter, at least about 5.0 mg per hour per liter, at least about 10 mg per hour per liter, at least about 15 mg per hour per liter, at least about 20.0 mg per hour per liter, at least about 25 mg per hour per liter, at least about 30 mg per hour per liter, at least about 50 mg per hour per liter, at least about 100 mg per hour per liter, at least about 200 mg per hour per liter, or at least about 500 mg per hour per liter.

[0117] Ethanol production can be measured using any method known in the art. For example, the quantity of ethanol in fermentation samples can be assessed using HPLC analysis. Many ethanol assay kits are commercially available that use, for example, alcohol oxidase enzyme based assays.

[0118] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

Example I—Trehalose Utilization

[0119] Expression cassettes for trehalose-6-P synthase (TPS1, SEQ ID NO: 9) and trehalose-6-P phosphatase (TPS2, SEQ ID NO: 10) from *Saccharomyces cerevisiae* were engineered into strain *S. cerevisiae* strain M12156 which contains glycerol reduction technology and expresses a glucoamylase. The cassettes were integrated at the IME1 locus, in a knock in fashion. The CYC1 terminator sequence was included downstream of the IME1 open reading frame (ORF) followed by the TPS1 and TPS2 expression cassettes which were driven by the promoters of TDH1 and PAU5 respectively. TDH1 is predicted to give strong constitutive expression of TPS1 whereas the PAU5 promoter has been shown to be induced by alcoholic fermentation and anaerobic conditions. The resulting strain was given the identifier M16807. The table below summarizes the genotype of the *Saccharomyces cerevisiae* strains used in this example.

TABLE 1

Genotype of <i>Saccharomyces cerevisiae</i> strains used in this example.			
Strain	Gene(s) overexpressed	Gene(s) inactivated	
M12156	STL1 (SEQ ID NO: 11) ADHE (SEQ ID NO: 15) PFLA (SEQ ID NO: 13) PFLB (SEQ ID NO: 14) GLU (SEQ ID NO: 16)	fdh1Δ fdh2Δ gpd2Δ	
M16807	Same as M12156 TPS1 (SEQ ID NO: 9) TPS2 (SEQ ID NO: 10)	Same as M12156	

[0120] The *Lactobacillus paracasei* strain 12A was engineered into an ethanologen by deletion of four native LDH enzymes coupled with the addition of the PDC (SEQ ID NO: 4) and ADHB (SEQ ID NO: 8 encoded by codon-optimized

SEQ ID NO: 6 and 7) enzymes from *Z. mobilis*. Two copies of the *Z. mobilis* genes (codon-optimized SEQ ID NO: 2 and 3) were integrated into the genome with one cassette driven by the glycolytic pgm promoter, and the second cassette driven by the promoter of the universal stress protein A (uspA) which has been shown to be up-regulated during late growth stages. In addition two native genes encoding mannitol-1-phosphate 5-dehydrogenase, mtlD1 and mtlD2, were also deleted to eliminate the conversion of fructose-6-phosphate to mannitol. The genotype of strain *Lactobacillus paracasei* used in this example is provided in Table 2.

TABLE 2

Genotype of <i>Lactobacillus paracasei</i> strain used in this example.			
Strain	Gene(s) overexpressed	Gene(s) inactivated	
12A	None - wild-type <i>Lactobacillus paracasei</i> parental strain		
M17744 (E3.1)	PDC (SEQ ID NO: 4) ADHB (SEQ ID NO: 8)	ldh1Δ, ldh2Δ, ldh3Δ, ldh4Δ mtlD1Δ, mtlD2Δ	

[0121] *S. cerevisiae* strains M12156 and M16807 were utilized to ferment commercial corn mash either with or without the inclusion of strain E3.1. Performance was characterized under standard commercial operating parameters (permissive) as well in the presence of high temperature stress. Fermentation parameters are outlined in Table 3 and metabolite concentrations were analyzed by HPLC following 50 hours of fermentation. As shown on FIG. 5, the results indicated that both M12156 and M16807 perform similarly under standard conditions either with or without the addition of E3.1. Conversely, when the strains underwent high temperature stress, M16807 produced significantly more ethanol than strain M12156 and had lower residual glucose at the end of fermentation. Likewise, co-fermentation with the ethanologen E3.1 also showed improved results for both M12156 and M16807 under stressful conditions. Most significantly, the combination of the new yeast strain M16807 with E3.1 had a synergistic effect showing higher ethanol titers than would be expected from the additive effects of trehalose biosynthesis and co-fermentation with E3.1.

TABLE 3

Fermentation parameters utilized to analyze performance in corn mash fermentation.				
	M12156	M12156 + E3.1	M16807	M16807 + E3.1
Yeast Dose gDCW/L	0.3	0.3	0.3	0.3
Bacterial Dose cfu/ml	N/A	1 × 10 ⁷	N/A	1 × 10 ⁷
% Total Solids	31.50%	31.50%	31.50%	31.50%
Spirizyme Excel GA Dose (AGU/gTS)	0.42	0.42	0.42	0.42
Urea ppm	300	300	300	300
Temperature 0-24 hours	33° C.	33° C.	35° C.	35° C.
Temperature 24-50 hours	31° C.	31° C.	33° C.	33° C.

Example II—Mannitol and Sorbitol Utilization

[0122] The sorbitol constructs included *Saccharomyces cerevisiae* M20043, which was constructed by introducing

4-copies (2-per chromosome) of the *E. coli* srlD, encoding sorbitol-6-phosphate dehydrogenase, into the fcyL locus of wild-type strain M2390. The corresponding engineered bacterium was *Lactobacillus paracasei* M19605, which was constructed from the ethanologen strain E3 (Δ L-ldh1:: P_{pgm} -PET, Δ L-ldh2, Δ D-hic, Δ mtlD1, Δ mtlD2, Δ L-ldh3PuspA-PET) by introduction of plasmid pDW2:: P_{31} -gutFCBA, which encode the sorbitol-6-phosphate dehydrogenase, and transporter subunits C, B, and A respectively.

[0123] The mannitol constructs were *Saccharomyces cerevisiae* M20036, which was engineered from M2390 by introducing 4-copies (two per chromosome) of the *Escherichia coli* mtlD, encoding mannitol-1-phosphate 5-dehydrogenase. The corresponding bacterium for this fermentation was *Lactobacillus paracasei* M19998, which was constructed from the ethanologen strain E3.1 (Δ L-ldh1:: P_{pgm} -PET, Δ L-ldh2, Δ D-hic, Δ mtlD1, Δ mtlD2, Δ L-ldh3PuspA-PET, Δ L-ldh4) by introduction of plasmid pDW2:: P_{31} -mtlIDCBA, which encode the mannitol-1-phosphate 5-dehydrogenase and transporter subunits C/B and A respectively.

[0124] Tables 4 and 5 summarize the genotypes of the yeast and bacterial host cells used in this Example.

TABLE 4

Genotype of <i>Saccharomyces cerevisiae</i> strains used in this example.		
Strain	Gene(s) overexpressed	Gene(s) inactivated
M2390	None - wild type parental strain used for M20043 and M20036	
M20043	SRLD (SEQ ID NO: 29)	fcyΔ
M20036	MTLD (SEQ ID NO: 35)	

TABLE 5

Genotype of <i>Lactobacillus paracasei</i> strain used in this example.		
Strain	Gene(s) overexpressed	Gene(s) inactivated
M19605	PDC (SEQ ID NO: 4) ADHB (SEQ ID NO: 8) GUTF (SEQ ID NO: 31) GUTC (SEQ ID NO: 33) GUTB (SEQ ID NO: 35) GUTA (SEQ ID NO: 37)	ldh1Δ, ldh2Δ, ldh3Δ, ldh4Δ mtlD1Δ, mtlD2Δ
M19998	PDC (SEQ ID NO: 4) ADHB (SEQ ID NO: 8) MTLD (SEQ ID NO: 27) MTLCB (SEQ ID NO: 41) MTLA (SEQ ID NO: 43)	ldh1Δ, ldh2Δ, ldh3Δ, ldh4Δ mtlD1Δ, mtlD2Δ

[0125] The engineered yeast and bacteria were grown individually or in combination in a modified chemically defined medium (mCDM) that contained the following components (per L): 2.0 g sodium citrate (2 H₂O), 1.0 g Potassium phosphate (mono basic), 1.0 g potassium phosphate (di basic), 200 mg sodium chloride, 200 mg calcium chloride (2 H₂O), 200 mg magnesium sulfate, 50 mg manganese sulfate, 1 mL Tween 80TM, 1 mL Tween 20TM, 1 mL glycerol, 10 μL mevalonolactone, 10 mg pyridoxal HCl, 20.0 mL RPMI 1640 vitamin solution, 10.0 g Bacto-casitone, 2.5 mg pyridoxamine dihydrochloride and 18 g Glucose (100 mM). All of the cell samples were washed twice with 0.85% saline, normalized to an OD₆₀₀ of 2.0 and inoculated at 0.1%. Samples were incubated at 35° C. for 67 hours, then the supernatant was collected and analyzed by HPLC.

[0126] As shown in FIGS. 6 and 7 as well as Table 6, the wild-type control strain of *Saccharomyces cerevisiae* (M2390) converted the glucose into 177.2 mM ethanol and 7.9 mM glycerol. As expected, fermentation of mCDM with the engineered yeast strains M20043 or M20036 alone led to reduced glycerol titers and slightly lower ethanol levels, as carbon was redirected from glycerol biosynthesis toward sorbitol or mannitol, respectively, in these hosts. Strain M20043 produced 4.2 mM sorbitol and decreased glycerol production by 45% compared to the wild-type yeast M2390. The mannitol-producing yeast M20036 accumulated 3.5 mM mannitol in the fermentate, and reduced glycerol levels by 35% compared to M2390 (Table 6).

[0127] Growth in mCDM by pure cultures of *Lactobacillus paracasei* ethanologens engineered to convert sorbitol (M19605) or mannitol (M19998) into ethanol contained lower levels of glycerol than was observed with individual yeast strains, and yielded ethanol levels that were similar to or slightly above results from single yeast (FIGS. 6 and 7 as well as Table 6).

[0128] In contrast, fermentations that were performed with yeast and bacteria pairs uniformly showed increased ethanol levels, even with the wild-type control yeast strain, M2390 (FIGS. 6 and 7 as well as Table 6). Co-fermentation with the sorbitol producing yeast M20043 and the sorbitol consuming bacterium M19605 enhanced ethanol yield by 2.9% over M2390 alone, compared to 1.6% when the bacterium was paired with M2390. As expected, the sorbitol observed in fermentations with M20043 alone was largely consumed when the yeast was paired with M19605. These data demonstrate the added yield obtained with M20043 and M19605 is the result of metabolic redirection of glycerol biosynthesis to ethanol (via sorbitol) by the co-engineered yeast and bacterium.

[0129] Co-fermentations with the mannitol producing yeast M20036 and the mannitol consuming bacterium M19998 showed a similar pattern. Ethanol production in the fermentation with co-engineered yeast and bacteria was 4.4% higher than M2390 alone, whereas a 2.8% increase was obtained when M19998 was paired with wild-type M2390. Once again, the mannitol that was present in fermentations with M20036 alone was essentially consumed when the yeast was paired with M19998. These data demonstrate the added yield obtained with M20036 and M19998 is the result of metabolic redirection of glycerol biosynthesis to ethanol (via mannitol) by the co-engineered yeast and bacterium.

TABLE 6

Final metabolite concentrations in mCDM fermented with yeast and bacteria strains co-engineered to redirect glycerol biosynthesis to ethanol.

Strain	Metabolite concentration (mM)				
	Glucose	Glycerol	Sorbitol	Mannitol	Ethanol
<i>S. cerevisiae</i> M2390	0	7.9	0	0	177.2
<i>S. cerevisiae</i> M20043	0	4.3	4.2	0	175.3
<i>S. cerevisiae</i> M20036	0	5.2	0	3.5	176.1
<i>L. paracasei</i> M19605	0	3.6	1.3	0	176.6
<i>L. paracasei</i> M19998	0	3.4	0	1.3	179.2
M2390 + M19605	0	4.8	0.5	0	180.2
M20043 + M19605	0	4.9	0.4	0	182.3
M2390 + M19998	0	5.8	0	0.4	182.3
M20036 + M19998	0	4.8	0	0.5	185.0

Example III—Acetate Utilization

[0130] Wild type strain *Saccharomyces cerevisiae* M8279 was engineered for acetate utilization by introducing 4-copies (2-per chromosome) of the *Bifidobacterium adolescentis* adhE and up-regulation of the ACS2 polypeptide (e.g., additional copies of the native gene (SEQ ID NO: 49) were included), encoding a bi-functional acetaldehyde/alcohol dehydrogenase and an acetyl-CoA synthetase respectively, at the ylr296W locus. In addition, 4-copies (2-per chromosome) of the heterologous NADP-specific alcohol dehydrogenase of *Entamoeba nuttalli* (e.g., having the amino acid sequence of SEQ ID NO: 45) was integrated at the apt2 locus. The presence of this enzyme increases the availability of cytosolic NADH, by creating a redox imbalance between glycolysis and ethanol fermentation, and increases acetate conversion in *S. cerevisiae*. As the introduced acetate conversion pathway is required to compete for NADH with the native glycerol biosynthetic pathway, the later was down regulated by deletion of gpd2, encoding a glycerol-3-phosphate dehydrogenase, and up-regulation of an heterologous glycerol transporter STL1 (from *P. sorbitophila*) resulting in the final yeast strain M10909.

TABLE 7

Genotype of <i>Saccharomyces cerevisiae</i> strains used in this example.		
Strain	Gene(s) overexpressed	Gene(s) inactivated
M8279	None - wild-type <i>Saccharomyces cerevisiae</i> parental strain	
M10909	STL1 (SEQ ID NO: 51) ADHE (SEQ ID NO: 15) ACS2 (SEQ ID NO: 49) NADP-specific alcohol dehydrogenase of <i>Entamoeba nuttalli</i> (SEQ ID NO: 45)	apt2Δ gpd2Δ

[0131] The engineered bacterium, M20896, is derived from the *Lactobacillus paracasei* strain 12A, which was converted to an ethanologen through deletion of four native lactate dehydrogenases, two native mannitol dehydrogenases, and incorporation of a heterologous production of ethanol cassette (PET) consisting of the *Zymomonas mobilis* pyruvate decarboxylase, and alcohol dehydrogenase (Δ L-Idh1::Pgpm-PET, Δ L-Idh2, Δ D-hic, Δ mtlD1, Δ mtlD2,

Δ L-Idh3PgspA-PET). No additional modifications were therefore made to the native citrate operon.

TABLE 8

Genotype of <i>Lactobacillus paracasei</i> strain used in this example.		
Strain	Gene(s) overexpressed	Gene(s) inactivated
12A	None - wild-type <i>Lactobacillus paracasei</i> parental strain	
M20896	PDC (SEQ ID NO: 4) ADHB (SEQ ID NO: 8)	ldh1Δ, ldh2Δ, ldh3Δ, ldh4Δ mtlD1Δ, mtlD2Δ
E5	PDC (SEQ ID NO: 4) ADHB (SEQ ID NO: 8)	ldh1Δ, ldh2Δ, ldh3Δ, ldh4Δ mtlD1Δ, mtlD2Δ

[0132] The engineered yeast and bacteria were grown individually or in combination in a modified chemically defined medium (mCDM) that contained either 50 or 100 mM glucose (e.g., for 1 L of mCDM: 2.0 g sodium citrate (2 H₂O), 1.0 g potassium phosphate (mono basic), 1.0 g potassium phosphate (di basic), 200 mg sodium chloride, 200 mg calcium chloride (2 H₂O), 200 mg magnesium sulfate, 50 mg manganese sulfate, 1 mL Tween™ 80, 1 mL Tween™ 20, 1 mL glycerol, 10 μL mevalonolactone, 10 mg pyridoxal HCl, 20.0 mL RPMI 1640 vitamin solution, 10.0 g bacto-casitone, 2.5 mg pyridoxamine dihydrochloride and 18 g glucose (100 mM) or 9 g Glucose (50 mM)). When indicated, sodium citrate was removed from the media preparation in order to determine the impact of citrate conversion on fermentation performance. The wild type yeast strain M8279 was also included in these experiments. All of the cell samples were washed 2x with 0.85% saline, normalized to an OD₆₀₀ of 2.0 and inoculated at 0.1%. Samples were incubated at 35° C. for 68 hours, then the supernatant was collected and analyzed by HPLC.

[0133] As shown in FIG. 9, the wild-type control strain 12A only consumed approximately 40% of available citrate when grown in mCDM (50 mM glucose) and consumed 11 mM of acetate. Conversely, E5, an ethanologen strain containing equivalent ethanol engineering as M20896 and differing only in their antimicrobial resistance profile, completely depleted citrate and generated acetate as a result (FIG. 9).

[0134] As shown in FIG. 10 and Table 9, the wild-type control strain of *Saccharomyces cerevisiae* (M8279) converted the glucose into 170.2 mM ethanol and 7.2 mM glycerol. As expected, fermentation of mCDM with the engineered yeast strains M10909 alone led to reduced glycerol titers and higher ethanol levels, as carbon was redirected from glycerol biosynthesis due to the down regulation of this pathway. Strain M10909 produced 6.7 mM glycerol and increased ethanol yield by 4.2% compared to the wild-type yeast M8279 (Table 9). Similarly, it was observed that co-fermentation with M20896 and M8279 led to a 2.4% yield increase over M8279 alone and a 35% reduction in glycerol titer.

TABLE 9

Strain	Metabolite concentration (mM)				
	Glucose	Glycerol	Acetate	Citrate	Ethanol
<i>S. cerevisiae</i> M8279 ¹	0.80	7.2	0.0	9.5	170.2
<i>S. cerevisiae</i> M10909 ²	1.44	6.7	0.0	9.6	176.2
<i>Lb. paracasei</i> M20896 ³	0.58	1.8	18.6	0.0	174.9
M8279 + M20896	0.42	4.0	20.2	0.0	175.0
M10909 + M20896	0.44	3.6	19.3	0.0	179.0

[0135] In contrast, when co-fermentations were performed utilizing both the engineered yeast and the bacterium pair, an overall ethanol yield increase was seen of 4.8% and a 50% reduction in glycerol titer was achieved (FIG. 11). This corresponded to a 3 mM increase in ethanol titer over M10909 alone while 1 mM of acetate was consumed.

[0136] While the invention has been described in connection with specific embodiments thereof, it will be understood that the scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

REFERENCES

- [0137] Peleg, A.Y., Hogan, D.A., Mylonakis, E., 2010. Medically important bacterial-fungal interactions. *Nat. Rev. Microbiol.* 8, 340-349.
- [0138] Schink, B., 2002. Synergistic interactions in the microbial world. *Antonie Van Leeuwenhoek* 81, 257-261.
- [0139] Wargo, M.J., Hogan, D. A., 2006. Fungal-bacterial interactions: a mixed bag of mingling microbes. *Curr. Opin. Microbiol.*, Host microbe interactions: fungi/Host microbe interactions: parasites/Host microbe interactions: viruses 9, 359-364.
- [0140] Yi, C., Wang, F., Dong, S., Li, H. 2016. Changes of trehalose content and expression of relative genes during the bioethanol fermentation by *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 62:827-835.
- [0141] U.S. Pat. No. 8,956,851
- [0142] WO 2012/138942
- [0143] WO 2011/153516
- [0144] WO 2017/037614
- [0145] WO 2015/023989
- [0146] WO 2018/013791

SEQUENCE LISTING

```

Sequence total quantity: 56
SEQ ID NO: 1      moltype = DNA length = 1707
FEATURE          Location/Qualifiers
source           1..1707
                 mol_type = other DNA
                 organism = Zymomonas mobilis

SEQUENCE: 1
atgagttata ctgtcggtac ctattnagcg gagcgggttg tccagattgg tctcaaggcat 60
cacttcgcag tcgcggggcga ctacaacctc gtccttggt acaaacctgct ttggaaacaaa 120
aacatggaggc aggttatttgc ctgttaacgaa ctgaactcgcg gtttcaagtgc agaagggttat 180
gtctcgccca aaggcgcgcgcg acgcggcgctc gttagccatca ggcgtcggtgc gctttccgcga 240
tttgcgtgtca tcgggtggcgc ctatgcggaa aaccctccgg ttatccgtat ctcgggtgtc 300
ccgaacaaca atgatcgcgc tgctgggtcac gtgttgcata acgcgttccgg caaaaccgcac 360
tatcaactatc agttggaaat ggccaagaac atcacggccg cagctgaagc gatttacacc 420
ccagaagaag ctccggctaa aatcgatcac gtgattaaaa ctgtcttcgc tgagaagaag 480
ccgggtttatc tcgaaatc ttgcacatc gtttccatgc cctgcggccgc ttctggacccg 540
gcaagcgcata tggtaatgc cgaaggccgcg gacgaaatgc ttgttgcatac agcgttgcac 600
gaaaccctgtaa aattcatcgc caaccgcgc acagggtccg ttctcgccgg cagcaagtcg 660
cgcgccgcgtc gtgtcgaaatc agctgtgtc aaatttgtctc atgtcttcgg tggcgccgtt 720
gttccatcg ctgtcgcaaa aacttccatc ccagaaggaaa accccgcattt catcggtacc 780
tcatgggggtt aagtgcgtca tccggggcgtt gaaaaggacgc tgaaaaggacgc cgatcggtt 840
atcgctctgg ctctgttcaatc caaccgcgtc tccaccactg gttggacccgaa tattctgtat 900
cctaagaaac tggttctcgca tgaaccgcgt tttgtcgatc ttaacggcgt tcgcttcccc 960
agcgttccatc tggaaatcata tttggccgtt tttgtcgatc aagttttccaa gaaaaccgggt 1020
gttccatcgatc ttttttttttccatc aatcgatcgtt gttggccgtt ttttttttttccatc 1080
ccggatgtctc cggttgtcaatc ccggatgttccatc aatcgatcgtt ttttttttttccatc 1140
aacacgcacgg ttattgtgtca aaccgggttca ttttttttttccatc aatcgatcgtt 1200
ccggatgttccatc ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1260
ggccgcgttccatc ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1320
ggttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1380
atcatcttc ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1440
tacaacaaca tcaagaactgttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1500
ggttatgtaca ggggttgttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1560
gctatcaagg ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1620
cgtaagact gcaactgttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1680
cgtaagccgttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt ttttttttttccatc aatcgatcgtt 1707

SEQ ID NO: 2      moltype = DNA length = 1707
FEATURE          Location/Qualifiers
misc_feature     1..1707
note = Codon optimized sequence of SEQ ID NO: 1
source           1..1707
                 mol_type = other DNA

```

-continued

```

organism = synthetic construct

SEQUENCE: 2
atgtcatata ccgttggcac ctattggct gaacgtttg ttcaaattcg cttgaagcac 60
cacttcgtc ttgcggcga ttataacttg gttttgttg ataacttgg tttcaacaaag 120
aacatggaa acatggaaat ctgcaacaa ttgaactcg ctttctcagc tgaaggctat 180
gtccgtgcta agggegctgc tgctgtgtt gttacattt cagttggcgc tttgtcagct 240
ttcgtatgta tcggggcgc ttatgtggaa aacttggcaag ttatctgtat ctcaggcgct 300
ccaaacaaca acgatcacgc tgctggccac gttttgacc acgcttggg caagaccat 360
tatcaactatc aattggaaat ggctaaagaac atcaccgtc ctgtgtaaac tatctataacc 420
ccagaagaag ctcccaqtaa gatecatcgtt gtttcaatggc cccgttggc tgaaaagaag 480
ccagtttattt tggaaatcgc tgcaacatc gtttcaatggc catggcgtc tccaggccca 540
gttccatgtt ttgttcaacgc tgaagcttca gatggactt catttgaacgc tgctgttggaa 600
gaaacacttga agttcatcgc taaccgtat aagggttgc ttttggttgg cttcaaggat 660
cgtgcgtcgtc gggctgttgaaga agtgcgtt aagttcgtat atgcgttggg cggcgctgtt 720
gttccatgtt ccgtgttcaaa gtttcaatggc cccgttggc tgaaaagaacata tateggcacc 780
tcatggggcc aagtttccata tccaggcgtt gaaaaggacca tgaaggaaac tgatgtgtt 840
atcgcttgg ctcccaqttt caacgattat tcaaccaccc gctggaccga tatcccacat 900
ccaaagaagaat tgggttggc tgaacccatc gtatgttgg ttaacggcgt tcgtttccca 960
tcagtccatc tgaaggatc ttggcttggc ttggctcaaa aggttcaaa gaagaccggc 1020
gttccatgtt ttgttcaatgc atttgcgtt ggcgttggc agaaggctgc tccaggctgtat 1080
ccatccatc catttggtaa cgctgttcaatc gctgtcaag ttgaaggctt gtttggccca 1140
aacaccaccc ttatcgctga aaccggcgt tcatgttca acgttcaacgc tatgaaggat 1200
ccaaacccgc ctcgtgttca atatggaaat caatggggcc acatccggcgt gtcgtttccca 1260
gttccatgtt gtatgttgc ttggcgttca acatgttggat ggttggcgat 1320
ggcttccatc aattggccgc tcaagaagtt gctcaatgg ttcgttggaa gtttccatgtt 1380
atcatcttct tcatgttcaaa ctatggctat accatcgaaat ttatgtatcca cgtatggccca 1440
tataaacaaca tcaagaactg ggatgtatg ggtttagtcaaa cggcaacccgc 1500
ggcttccatc caggcgctgg taaggcttgc aaggcttgc cccggccgcg attggctgaa 1560
gttccatgtt ttgttggc ttaaccggat gggcccaacct tcatgttcaaa ttcatgttgc 1620
cgtgaagatc gacccgaaat ttgggttcaaa tggggcaacgc gtttggctgc tgcttcaactca 1680
cgtaaggccat ttaacaatgtt gtttggat 1707

SEQ ID NO: 3      moltype = DNA length = 1707
FEATURE          Location/Qualifiers
misc_feature    1..1707
note = Codon optimized sequence of SEQ ID NO :1
source           1..1707
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 3
atgagctaca ctgttggtac ttacttagct gaacgcttag ttcagatcg tttaaaggcat 60
cattttgtc ttgcagggtga ttacaacttta gttttatttag ataacttattt attaaacaag 120
aatatggaa acatggaaat ttgttcaacgc tttaacttgc gtttcaacgcg agaaagggtac 180
gttccatgtt ggggttgc tgcaggcttgc gtttcaatggc ttatgttgc ttttcaaggat 240
tttccatgtt ccgtgttcaaa aatttaccat ttatgttgc ttttcaatggc ttatgttgc 300
ccaaacaaca atgaccatgc tgcaggctat gtttacatc atgttggat taagactgtat 360
taccatattc aatttggaaat ggcaaaaggaaat attactgttgc ctgcaggaaac tatttacat 420
ctgcaggaaat caccaggatc aatttgcgtt gtttcaatggc ttatgttgc cgaaaaggaaa 480
ctgttccatc tagaaattgc ttgttcaatc gtttcaatggc ttatgttgc accaggccca 540
gttccatgtt tatttgc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 600
gaaatcttgc aatttgcgtt gtttcaatggc ttatgttgc gtttcaatggc ttatgttgc 660
cgccgttgc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 720
gcaactatgc ctgcaggatc gtttcaatggc ttatgttgc ttttcaatggc ttatgttgc 780
tccctgggtt gtttcaatggc ttatgttgc gtttcaatggc ttatgttgc 840
atttgcgttcc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 900
ccaaagaat ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 960
agcggttccatc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1020
gttccatgtt ttgttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1080
ccatctgtc ttatgttgc gtttcaatggc ttatgttgc 1140
aacactactg ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1200
ccaaacccgttgc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1260
gttccatgtt ccgtgttcaaa ctttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1320
ggtagcttcc agtttactgc ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1380
attatcttct ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1440
tacaacaata ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1500
ggtttccatc ctttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1560
gcaatcaaaat ttttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1620
cgtgaagact gtttcaatggc ttatgttgc gtttcaatggc ttatgttgc 1680
cgtaaaccat ttttcaatggc ttatgttgc 1707

SEQ ID NO: 4      moltype = AA length = 568
FEATURE          Location/Qualifiers
source           1..568
mol_type = protein
organism = Zymomonas mobilis

SEQUENCE: 4

```

-continued

MSVTGVTYLA	ERLVQIGLKH	HFAVAGDYNL	VLLDNLLNPK	NMEQVYCCNE	LNCGFSAEYG	60
ARAKGAAAAAV	VTYSGALSA	FDAIGGAYAE	NLPVILISGA	PNNNDHAAGH	VLHHALGKTD	120
YHQLEAKM	ITAAABAIYT	PEEAPAKIDH	VIKTALEREKK	PVYLETACNI	ASMPCAAPGP	180
ASALFNDEAS	DEASLNAAVE	ETLKFIANRD	KVAVLVGSKL	RAAGAEEAAV	KFADALGGVV	240
ATMAAKSF	PEENPHYIQT	SGWEFVSYPG	EKTMKHEADA	IALAPVNNDY	STTGTWTDIPD	300
PKKLVLAEP	SVVNVGVRFP	SVHLKDYLTR	LAQKVSKKTG	ALDFFKSLNA	GELKKAAPAD	360
PSAPLVNAEI	ARQVEALLTP	NTTVIAETGD	SWFNAQRMLK	PNGARVEYEM	QWGHHIGWSVP	420
AAFGYAVGAG	ERRNILMVGD	GSFQLTAQEY	AQMVRKLKPV	IIFLINNYGY	TIEVMIHDP	480
YNNIKNWDYA	GLMEVFNGN	GYDSGAGKGL	AKTGGELAE	AIKVALANTD	GPTLIECFIG	540
REDCTEELVK	WGKRVAAGANS	RKPVNKLL				568

```

SEQ ID NO: 5          moltype = DNA  length = 1152
FEATURE              Location/Qualifiers
source               1..1152
                     mol_type = other DNA
                     organism = Zymomonas mobilis

SEQUENCE: 5
atggcttctt caactttta tattccttc gtcaacgaaa tgggcgaagg ttgcgttcaa 60
aaagcaatca aggatctta cggcagcggc tttaaaaaatcg cgctgtatcg ttctgtatcg 120
ttcatgaaca aatccggtgt tgtgaacgag gttgtcgacc ttgttggaaagg acagggttt 180
aattctgtctt tttatgtgg cggttatgcgg aaccgcactg ttaccgcagt tcttggaaaggc 240
cttaagatccc tgaaggatcaa caattcagac ttgcgtatct ccctcgttgg tggttctccc 300
catgactgcg ccaaaggccat cgctctgtgc gcaaccatcg gtgggtgaagg ccaaagactac 360
aaaggatctatc aacaatctaa gaaacctggc ctgccttgg tgcgttcaatcaa cacgacggct 420
ggtagccgtt ctgaaatgtac ggcttctgc atccatctgt atggatccgc tcacgtttaag 480
atggccatttggtgcgtca cggttaccggc atgggttcccg tcaacgcattcc tctgttgcgt 540
gttggatgtccaaaggccgttgcgtcc acccgatgg atgcgttgcgtcc accgcgttgcgt 600
gaaggettattcttcaacggc agetacttccg atcaacgcattcc tttgcgttgcgtcc gaaggcttgcg 660
ttcatgtatccctaagaatcttca aagaggccatcg tgcgttgcgtcc ttgttggatgcgcgttgcgt 720
gaaggtatggctttagccatccatctgtcc ggtatggcttgcgttgcgtcc ttgttggatgcgcgttgcgt 780
ttatgtccatctatgttgcgtcc aacgttgcgtcc ggttacttacaa accttgcgtcc tttgttgcgtcc 840
aaaggctgttgcgttgcgtcc tttgttgcgtcc ttgttgcgtcc tttgttgcgtcc tttgttgcgtcc 900
aaagaggcttgcgttgcgtcc tttgttgcgtcc atccatctgtcc tttgttgcgtcc tttgttgcgtcc 960
gaaggccatccatctgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc 1020
accggatgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc 1080
gttggatgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc 1140
aaaggccatccatctgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc tttgttgcgtcc 1200

```

```
SEQ ID NO: 6          moltype = DNA  length = 1152
FEATURE
misc_feature          Location/Qualifiers
                      1..1152
note = Codon optimized sequence of SEQ ID NO: 5
source               1..1152
                     mol_type = other DNA
                     organism = synthetic construct
```

SEQUENCE:	Organism	6				
atggcttcat	caacccattta	tatccccattc	gttaaacgaaa	tggggcagg	ctcatggaa	60
aaggctatca	aggatgttggaa	cggtccgcgc	ttcaaaacg	ctttgatct	ttcacatgt	120
ttcataaca	agtccaggcgt	tgtttaagcaa	gttgcgttgc	tgtttaaggc	tcaaggcata	180
aactcagtcg	tttatgttgg	cgttataccgc	aaccaccaac	ttaaccctgt	tttggaaaggc	240
ttgaagatct	tgaaggatcaa	caactcaagat	ttcgatgtatct	cattggggcg	cggtccacca	300
cacgttgcg	ctaaggctat	cgcttgcgtt	gctaccacac	cgccggcaga	taaggattat	360
gaaggccatcg	ataagtcaaa	gaaggccatgt	ttgcattgttgc	tgttcaatcaa	caccaccgt	420
ggcacccgtt	cagaatgtac	cggtttgc	atcatacaccg	atgaatgtcg	tcacgtttaag	480
atggctatcg	ttgatcgta	cgttacccca	atggtttgc	ttaacgatcc	attgttgcgt	540
gttggcatgc	caaagggtt	gaccgtcg	accggcatgg	atgttgcgt	ccacgcttgc	600
gaagcttatt	catcaacccgc	tgcataccccc	atcaccggat	cttgcgttgc	gaaggctgtgc	660
tcataatgtcg	ctaagaacct	gaagacccgt	tgcgatataacg	gcaagatata	ggccacgtcg	720
gaagctatgg	ctttagtgc	attcttgcgt	ggcatggctt	tcaacaacgc	ttcatgtggc	780
tatgttccac	ctatgtgtcc	ccaaatgggc	ggctatattata	acttgcacca	cggcggttgc	840
aaaggctgtt	tgttgtccaca	cgtttttgttgc	tataacgcgtt	cgttttgtc	ttggccgttgc	900
aaggatgttgc	gggttgcata	gggttgcgtat	atcgatcaact	tggggcataa	ggaaggcgct	960
gaagctaccat	tccaaagctgt	tcgtatgttgc	gctgcgttcaa	tcggcatccc	agctaacttgc	1020
accgaatgttgc	gggttgcata	gggttgcgtat	ccattttgttgc	ctgtatcacgc	tttggaaaggat	1080
gtttgcgttgc	tgaccaccc	acgttcaaggc	gatcaaaagg	aagttaaga	attgttgcgttgc	1140
tcatacttttca	aa					1152

-continued

aaggcaatta	aggatttaaa	cggttcaggt	ttaagaacg	cattaatcgt	tagcgatgc	120
ttcatgaata	agagecggtg	tgtaaacag	gttgctgact	tataaaaggc	acaaggatc	180
aacagcgtg	tttacgatgg	tgttatgc	aacccaactg	ttactgtgt	tttagaaaggt	240
ttaaagattt	taaaggacaa	caacagcgc	ttcggttattt	cattaggtgg	ttggtcacca	300
catgattgtg	ctaaggcaat	cgcattagtt	gcaactaacg	gtggtaagt	taaagattac	360
gaaggatcg	acaaggacaa	gaagocgtgt	ttaccattaa	tgagcatcaa	cactactgt	420
ggtaactgtca	gcgaaatgc	tcgtttctgt	atcatcactg	acgaagtccg	ccatgttaaa	480
atggcaattt	ttgaccgtca	tgttactctt	atggttagc	ttAACGACCC	attattatg	540
gttggatgtc	ctaagggttt	aactgctgt	actggatgtgg	acgtttaac	tcatgcattc	600
gaagcatact	catcaactgc	tgcaactcca	attachactt	cttgtgttgg	aaaggcagct	660
agcatgtatcg	caaaggatcg	aaagactgtt	tgtgataacg	gttaggcacat	gcctgcacgt	720
gaagcaatgg	tttacgctca	gttcttagt	ggtagggcat	tcaataaacgc	tagtttagt	780
taclgttcatg	caatggcaca	tcagtttagt	ggttactaca	acttaccaca	ttggtttgc	840
aatgtctgtc	tgtttaccca	tgtttagtgc	tacaacgcgt	gcgttgcgtc	aggcggttta	900
aaggacgttg	tgtttgcgt	gggtttagac	atcgcaaaact	tagtgacaa	ggaagggtgt	960
gaagcaacta	ttcaggcaqt	tcgtgtacta	gctgtgtca	tcggtatccc	tgtaacttta	1020
actgaattag	gtgcaaaagaa	ggaagacgtt	cctttattag	ctgaccatgc	tttaaaggac	1080
gttggatgtt	taactaaccc	tcgtcaaggt	gatcagaaag	aagtcaaga	attattctt	1140
agcgattctt	aa					1152

SEQ ID NO: 8	moltype = AA	length = 383			
FEATURE	Location/Qualifiers				
source	1..383				
	mol_type = protein				
	organism = Zymomonas mobilis				
SEQUENCE: 8					
MASSTFYIIPF VNEMGEGSLE	KAIKDLNGSG	FKNALIVSDA	FMNKSGVVKQ	VADLLKAQGI	60
NSAVYDGMP NPTVTAVLEG	LKILKDNNSD	FVISLGGSSP	HDCAKIALV	ATNGGEVKDY	120
EGIDKSKKPA LPLMSINTTA	GTASEMTRFC	IITDEVRHVK	MAIVDRHRVTP	MVSVDPLLM	180
VGMPKGTLAA TGMDALTHAF	EAYSSSTAATP	ITDACLKAA	SMAIKNLKTA	CDNGKDMMPAR	240
EAMAYAQFLA GMAFNNASLG	YVHAMAHQLG	GYYNLPHGVLC	NAVLLPHVLA	YNASVVAAGR	300
KDVGVAMGLD IANLGDKEGA	EATIQAVRDL	AASIGIPANL	TELGAKEEDV	PLLADHALKD	360
ACALTNPRQG DQKEVEELFL	SAF				383

SEQ ID NO: 9	moltype = AA	length = 495
FEATURE	Location/Qualifiers	
source	1..495	
	mol_type = protein	
	organism = Saccharomyces cerevisiae	
SEQUENCE: 9		

MTTDDNAKQL TSSSGGNIIV	VSNRLPVIT	KNSSTGQYEY	AMSSGGLVTA	LEGLKKTYTF	60
KWPGWPGLEI	PDEKDQVRK	DLEKFNAVP	IFLSDEIADL	HYNGFNSIL	120
INFDENAWLA	YNEANQFTFH	EIAKTMHNHD	LIWVHDYHLM	LPPEMLRVKI	180
GWFLHTPFL	SEIYRILPVR	DLVGFHTYDY	ARHFLSSVQR	VLNVNTPPNG	240
VEYQGRFVN	GAFFIGIDV	KFTDGLKKE	VQKRIQQQLKE	TFKGCKIIVG	300
PQKLHAMEVF	LNEHPEWRKG	VVLVQVAVPS	RGDVEEYQYL	RSVNVNELVGR	360
VP1HFHMHKSI	PFEELISLYA	VSDVCLVSVT	RDGMNLVSYE	YIACQEKKKG	420
AQSLNGAIIV	NPWNTDDLS	AINEALTPD	VKEVNWKEI	YKYISKYTS	480
YSTSSSSSTSS	SATKN				495

SEQ ID NO: 10	moltype = AA	length = 896
FEATURE	Location/Qualifiers	
source	1..896	
	mol_type = protein	
	organism = Saccharomyces cerevisiae	
SEQUENCE: 10		

MTTTAQDNSP KKRQRIIINCV	TQLPYKIOLG	ESNDWDKISA	TTGNSALYSS	LEYLQFDSTE	60
YEQHVVWTG	EITRTERNLF	TREAKEKPQD	LDDDPLYLT	EQINGLTTT	120
KTDTTQTAPV	TNNVHPVWLL	RKNQSRWRNY	AEKVIWPTFH	YILNPNSEGE	180
KFNEAYAQKII	WIHDYLLL	PQLLRMKFND	ESIIIGYFHH	APWPSNEYFR	240
CLPRRKQILD	GLVGNARICP	QNESFSRHFV	SSCKRLLDAT	AKKSNSNSDS	300
DVLVDSLPIG	VNTTQILKDA	FTKIDDSKVL	SIKQAYQNKK	IIIGRDRLLS	360
FETFLAMYPE	WRDQVVLQIV	SSPTANRNSP	QTIRLEQQVN	ELVNSINSSEY	420
YYMRIPKDVY	LSLLRVADLC	LITSVRDGMN	TTALEYVTVK	SHMSNFCLCYG	480
SSNVLKDAIV	VNPWDWSAVA	K\$INMALKLD	KEEKSNLLESK	NPLILSEFSG	540
EQASSDDDVE	RKMTPALNR	VLLENYKQAK	RRFLFLDYDG	TLTPIVKDPAAI	600
ILQKLCADPH	NQIWIISGRD	QKFLNWKLLG	KLPQLGLSAE	HGCFCMKDVSC	660
DMSWQVRVNE	VMEEFTTRTP	GSFIERKKVA	LTHYRRTVPE	ELGEFHAKEL	720
FDLEVMDGKA	NIEVRPRFVN	KGEIVKRLVW	HQHGKPQDML	KGISEKLPKD	780
DDFTDEDMFR	QLNTIETCWK	EKYPDQKNQW	GNYGFYPVTV	GSASKKTVA	840
ETLGLLVGDV	SLFQSAGTV	LDSRGHVKN	ESSLKSCLAS	KAYVMRSAS	896

SEQ ID NO: 11	moltype = AA	length = 569
FEATURE	Location/Qualifiers	
source	1..569	
	mol_type = protein	

-continued

```

organism = Saccharomyces cerevisiae

SEQUENCE: 11
MIDLKLSNFK GKFISRTSHW GLTGKKLRYF ITIASMTGFS LFGYDQGLMA SLITGKQFNY 60
EFFPATKENGD HDRHATVVGQ ATTSCYELGC FAGSLFVMFC GERIGRKPLI LMGSVITIIG 120
AVISTCAFRG YWALGQFIIG RVVTGVTGGL NTSTIPWQOS EMSKAENRGL LVNLEGSTIA 180
FGTMIAWID PGLSYTNSSV QWRFPVSMQI VFALFLAAM IKLPESPRLW ISQSRTEEAR 240
YLVGTLDAD PNDEEVITEV AMLHDAVNRT KHEKHSLSLSS RSRGRSQQNLQ RALIAASTQF 300
FQQFTGCNAAS IYYSTVLFNK TIKLDYRLSM IIIGGVFATIY ALSTIGSFEL IEKLGRRKLF 360
LLGATGQAVS FTITFACLVK ENKENARGAA VGLFLFITFF GLSLLSLPWI YPPEIASMKV 420
RASTNAFSTC TNWLNCFAVV MFTFPIFIGQS GWGCYLFFAV MNLYIPVII FFYPETAGRS 480
LEBIDIIFAK AYEDGTOPWV VANHLPKLSL QVEFDHANAL GSYDDEMEKE DFGEDRVEDT 540
YNNQINGDNSS SSSNIKNEDT VNNDKANFEG 569

SEQ ID NO: 12      moltype = AA length = 440
FEATURE          Location/Qualifiers
source           1..440
mol_type = protein
organism = Saccharomyces cerevisiae

SEQUENCE: 12
MLAVRRILTRY TFLKRTHPVL YTRRAYKILP SRSTFLRRSL LQTQLHSKMT AHTNIQKH 60
CHEDHPIRRDS DSAVSIVHLK RAPFKVTIG SGNWGTIAK VIAENTELHS HIFEPEVRMW 120
VFDEKIGDEN LTDIINTRHQ NVKYLVPNIDL PHNLVADPDL LHSIKGADIL VFNIPHQFLP 180
NIVKQLQGHV AVPHVRAISCL KGFELGSKGV QLSSSYVTDT LGIQCAGLSG ANLAPEVAK 240
HWSETTVAYQ LPKDYGQGDGE DVHDHKLLL FHRPYFHVN VIDDVAGISIA GALKNVVALA 300
CGFVEGMGWG NNASAAIQRL GLGEIICKFGR MFFPESKVET YYQESAGVAD LITTCSGRN 360
VKVATYMAKT CKSALEAEKE LLNGQSAQGI ITCREVHEWL QTCELTQEFP LFEAVYQIVY 420
NNVRMEDLPE MIEELDIDDE 440

SEQ ID NO: 13      moltype = AA length = 292
FEATURE          Location/Qualifiers
source           1..292
mol_type = protein
organism = Bifidobacterium adoloscentis

SEQUENCE: 13
MSEHIFRSTT RHMLRDSKD VNZTLMGGLS GFESPIGLDR LDRIKALKSG DIGFVHSWDI 60
NTSVDGPCTR MTVFMMSGCP RLQYCQNPDW WKMRDGPKVY YEAMVKKIER YADLFKATGG 120
GITFSGGESM MQPAFVSRVF HAAKQMGVHT CLDTSGFLGA SYTDDMVDDI DLCLLDVKG 180
DEETYHKVTG GILQPTIDFG QRLAKAGKKI WVRFVLVPGL TSSEENVENV AKICETFGDA 240
LEHIDVLPFH QLGRPKWHML NIPYPLEDDQK GPSAAMKQRV VEQFQSHGFT VY 292

SEQ ID NO: 14      moltype = AA length = 791
FEATURE          Location/Qualifiers
source           1..791
mol_type = protein
organism = Bifidobacterium adoloscentis

SEQUENCE: 14
MAAVDATAVS QEELEAKAWE GFTEGNWQKD IDVRDFIQLN YTPYEDESF LADATDKTKH 60
LWKYLDDNYL SVERKQRVYD VDTHTPAGID AFPAGYIDSP EVDNVIVGLQ TDVPCRKAMM 120
PNNGWWRMVEQ AIKEAGKEPD PEIKKIFTKY RKTHNDGVFG VTOKQIKVAR HNKILTGLPD 180
AYGRGRIGD YRRVALYGVN ALIKFKQRDK DSIPYRNDFT EPEIEHWIRF REEHDEQIKA 240
LKQLINLGNE YGLDLSRPAQ TAQEAVQWLTY MGYLASVKSQ DGAAMSFGRV STFFDVYFER 300
DLKAGKITEA DAQEIIDNLV MKLRLRVRFL TKDYDAIFNLV DPYWATWSDA GFGDDGRTMV 360
TKTSFRLLNT LTLEHLGPGE EPNITIFWDP KLPPEAKRKC ARISIDTSAI QYESDKEIRS 420
HWGDDAAIAC CVSPMRVGKQ MQFFAARVNS AKALLYAING GRDEMTGMQV IDKGVIDPIK 480
PEADGTLDYE KVVKANYEKAL EWLSETYVMA LNIIHMHDX YAYESIEMAL HDKEVYRTL 540
CGMSGLSIAA DLSLACKYAK VPIYINNDKQ TTPGHENEYV EGADDLIVG YRTEGDFPLY 600
GNDDDRADDI AKWVSTVVMG QVKRPLVYRD AVPTQSILTI TSNVEYKAT GAFPSGHKKG 660
TPYAPGANPE NGMDSHGMLP SMFSVKGIDY NDALDGISLT NTITPDGLGR DEEERIGNLV 720
GILDAGNHG LYHANINVLR KEQLEDAVEH PEKYPHLLTVR VSGYAVNFVK LTKEQQLDVI 780
SRTFHQGAVV D 791

SEQ ID NO: 15      moltype = AA length = 910
FEATURE          Location/Qualifiers
source           1..910
mol_type = protein
organism = Bifidobacterium adoloscentis

SEQUENCE: 15
MADAKKKEEP TKPTPEEKLA AAEAEVDALV KKGLKALDEF EKLDQKQVDH IVAKASVAAL 60
NIKHLVLAQMA VEETHRGLVE DKATKNIFAC EHVTNYLAGQ KTVGIIREDD VLGIDEIAEP 120
VGVAGVTPV TNPTSTAIFK SLIALKTRCP IIIFGFHPGAQ NCSVAAAKIV RDAAIAAGAP 180
ENCIQWIEHP SIEATGALMK HDGVATILAT GGPGMVKAAY SSGKPALGVG AGNAPAYVDK 240
NVDVVRRAAND LILSKHFDYQ MIMATEQAIID ADKDIYAPLV KELKRRKAYF VNADEKAKLE 300
QYMFHGCTAYS GQTPKLNSSV PGKSPQYIAK AAGFEIPEDA TILAECKEV GENEPLTMEK 360
LAPVQAVLKS DNKEQAFEMC EAMLKHGAGH TAAIHTNDRD LVREYQORMH ACRIIWNSPS 420
SLGGVGDIYN AIAPSLLGC GSYGGNSVSG NVQAVNLINI KRIARRNNNM QWFKIPAKTY 480
FEPNAIKYLR DMYGIEKAVI VCDKVMEQLG IVDKIIDQLR ARSNRVTFRI IDYVEPEPSV 540

```

-continued

ETVERGAAMM	REEFEPDTII	AVGGGSPMDA	SKIMWLLYEH	PEISFSVDRE	KFFDIRKRAF	600
KIPPLGKKK	LVCIPITSSGT	GSEVTPFAVI	TDHKTGYKYP	ITDYALTPSV	AIVDPVLART	660
QPRKLASDAG	FDALTHAFEA	YVSVYANDFT	DGMALHAAKL	VWDNLAESVN	GEPEEEKTRA	720
QEKMHNAAATM	AGMAFGSAFL	GMCHGMAHTI	GALCHVAHGR	TNSILLPYVI	RYNGSVPEEP	780
TSWPKYNKYI	APERYQEIAK	NLGVNPGKTP	EEGVENLAKA	VEDYRDNKLG	MNKSFQECGV	840
DEDYYWSIID	QIGMRAYEDQ	CAPANPRIPO	IEDMKDIAIA	AYYGVSQAEG	HKLRVQRQGE	900
AATEEASERA						910

SEQ ID NO: 16	moltype = AA	length = 515
FEATURE	Location/Qualifiers	
source	1..515	
	mol_type = protein	
	organism = Saccharomyces fibuligera	

SEQUENCE: 16						
MIRLTVFLTA	VFAAVASCVP	VELDKRNTGH	FQAYSGYTVRA	RSNFTQWIHE	QPAVSWYLL	60
QNIIDYPEQOF	KAQKPGVVVA	SPSTSEPDYF	YOWTRDTAIT	FLSLIAEVED	HSFSNTTLAK	120
VVEYYISNTY	TLQRVSNSPG	NFDSPNHDL	GEPKFNVDDT	AYTASWGRPQ	NDGPALRAYA	180
ISRYLNAVAK	HNNKGKLLLAG	QNGIPIVSSAS	DIYWKIIKPD	LQHVSTHWST	SGFDLWEENO	240
GTHFFTALVQ	LKALSYGIP	SKTYNDPGFT	SWLEKQKDAL	NSYINSSGFV	NSGKKHIVES	300
PQLSSRGGLD	SATYIALIT	HDIGDDDTYT	PFNVDNSYVL	NSLYYLLVDN	KNRYKINGNY	360
KAGAAVGGRYP	EDVYNGVGTS	EGNPWQLATA	YAGQTFYTLA	YNSLKNKKNL	VIEKLNNDLY	420
NSPIADLSKI	DSSYASKDSL	TLYTGSNDYK	NVIKSLLQFG	DSFLKVLLDH	IDDNGQLEE	480
INRYTGFQAG	AVSLTWSSGS	LLSANRARNK	LIELL			515

SEQ ID NO: 17	moltype = AA	length = 510
FEATURE	Location/Qualifiers	
source	1..510	
	mol_type = protein	
	organism = Lactobacillus paracasei	

SEQUENCE: 17						
MVKNTLNLDI	PEPYADQYGV	YGGEFANIKP	YDEHARHINP	VKPDHSKLVA	SIHDAIVATG	60
LKDGMTISFH	HHFREGDYVM	NMVLAIEIAKM	GIKNLSTAPS	SIANVHEPLI	EHIKNGVVTN	120
ITSSGLRDKV	GAAISSGIMK	NPVVIRSHGG	RARAIARGDI	HIDVAFLGAP	SSDEYGNING	180
TKGKATCGSL	GYAMIDAKYA	DQVVAITDSL	MPYPNTPISI	PQTDVYVVQ	VDAIGDPTGI	240
AKGATRFTKN	PKELKIAEYA	AEVITKSAYF	KNGFSFQTGT	GGSSLAVARF	LRQAMLDQDI	300
KASFALGGIT	NSMVELLKEG	LVEKIIDVQD	FDHPSSAVSLG	ENADHYEIDA	NMYASPLSKG	360
AVINQLDIAI	LSALEIDTNF	NVNVITGSDG	IIRGASGGHS	DTSAACKMSM	VIAPLVRGRI	420
PTIVENVNTV	VTPGASVDDVV	VTEVGVAINP	ARTDLIEMFK	NLKVPFLSIE	DLKKMAYQIT	480
GTPEAIEYGD	KVALIEYRD	GTLIDVVHN				510

SEQ ID NO: 18	moltype = DNA	length = 1533
FEATURE	Location/Qualifiers	
source	1..1533	
	mol_type = other DNA	
	organism = Lactobacillus paracasei	

SEQUENCE: 18						
atggtaaga	atacactcaa	ccgtatata	ccagaaccat	atgcggatca	atacggttgtt	60
tatggccg	cgatggccaa	cattaagct	ttatgcgaat	atgcccgc	catcaatccg	120
gttaaggccg	atcacagcaa	actctggcg	tcaatttcac	atgccatgtt	agcaacttgg	180
ctgaaggacg	gcatgaccat	ttcttttac	catcatttt	gtgaagggg	ctatgtatg	240
aacatggcc	tagctggat	tgcggaaaatg	gggatcaaga	acctgtcaat	tgcccaact	300
tcgattggca	atgtatcatg	accattgatt	gagcacatca	aaaacgggt	gtgtaccaac	360
atcaccagtt	ccggcttgcg	cgacaaaatg	ggggcagca	tttcaagccg	catcatgaag	420
aatccagtt	tgattcgtc	acatggcg	cgccccgag	ccattgctcg	tggcgatatt	480
catattgcg	ttgccttct	ttggccccc	agcagtatgt	agtaacggca	cattaaacggc	540
acaaaaaggta	aggcgacctg	tggctgtta	gggtatgcga	tgattgcgc	aaaaatatgcg	600
gatcaagttg	ttgcattac	tgacgttta	atgccccat	cgatacggcc	aatcagcatt	660
ccgcaaaaccc	acgttgacta	tgtctgtca	gttgatgcga	ttggcgatcc	aactggatt	720
gccaaagggt	cgaccctgtt	cacaaagaac	ccgaaggaa	taaaaattgc	ggagtatgcg	780
gcagagggtca	tttaccaatc	ggcctactt	aaaaatgggt	tctcattcca	gaccggta	840
ggccggcttt	cgctggctgt	ttcgccgttt	ctgcggca	cgatgttgg	ccaaagacatc	900
aaagcttagt	ttgccttggg	ttgcattacc	aattcaatgg	ttgaatttt	gaaggaaaggc	960
cttgcgaaa	agattatcga	tgtcgaggac	tttgaccatc	cctctcgccgt	ttcatttaggc	1020
gagaacgcg	atcattacg	gattgtatgt	aatatgtacg	cgtcacccgtt	aagcaaagg	1080
gcagttatca	atcgtttaga	ttatgcgtt	ttatcggcac	tggaaatttg	tactaactt	1140
aacgttaacg	tgatcacggg	ttctgacggc	attatccgt	gctgttccg	tggccatagt	1200
gacacaacgt	ctgcctgca	aatgagcatg	gtgattgcgc	cactgttccg	cggtcggtatc	1260
ccaaacgtt	ttgaaaatgt	caatactgtt	gtgacaccgg	gtgccagtgt	tgacgttgc	1320
gtgaccgcg	tcggcgctcg	tattaatcca	gcacggactt	atgtttaaa		1380
aatctgaaag	tcggcgctgtt	ttcaattgaa	gatctgaaa	agatggcc	tcaaattact	1440
ggtagcaccgg	aagccatcga	atatggcgt	aaatgttgcg	cttgcgtca	atatcgcgt	1500
ggccacccgtt	tcgtatgttgt	tcacaatgtt	taa			1533

SEQ ID NO: 19	moltype = AA	length = 292
FEATURE	Location/Qualifiers	
source	1..292	

-continued

```

mol_type = protein
organism = Lactobacillus paracasei

SEQUENCE: 19
MDKLRRTMMF VPGANPGMLR DAPIYGADAI MFDLEDAVSL KEKDTARMLV YSALKTFDYS 60
SVETVVVRNA LDAGGDQDIE AMVLGGINVV RLPKTETAQD IIDVDAVITA VEEKYGIQNG 120
TTHMMAIES AEGVLNAREI AQASSRMIGI ALGAEDYLTG QHTHRSTDGA ELSFARNYIL 180
HAAREAGIAA IDTVYTQVDN EEEGLRHETAL IKQLGFEDGKS VINPRQIPVI NGVFAPALAE 240
VQKAREIVAG LKEAEAKGAG VVSVNGQMVDP KPVVERAQYT IALAKASGME 292

SEQ ID NO: 20      moltype = DNA length = 879
FEATURE           Location/Qualifiers
source            1..879
                  mol_type = other DNA
                  organism = Lactobacillus paracasei

SEQUENCE: 20
atgataaat taagaagaac catgatgtt gtgcctggc ccaatccggg catgttacgt 60
gatgctcga tttatgggtc tgatgcgatc atgtttgc ttgaagatgc tgtttcttg 120
aaggaaaaaa acacggcgcg aatgttgcgatt tattcagccc tgaagaccc ttgattacagt 180
agcgtggaaa cagttgtgcg ggtatgcgatc tttagatgcg cggggatca agacattgaa 240
gcgcgttgc ttggcgccat taatgttgcgatc cgcgttgc cgcgttgc ccaaaac tgcgttacgt 300
attattgtat ttgtatgtgtt catcacagca gttgaagaga agtacggcat tcaaaatggc 360
accacgcaca tgatggctgc aatttgcgtcg gctgaagggg ttttgaatgc tcgcgaaatc 420
gcacatcaatc catcaatgtt gattgggtt gcgttgggtt cagaagatc tctgcgatc 480
caacatacc accgcgttcgcgatc gatgttgcgatc ttggccgtt cttatctcg 540
catgcgtgcg gagaagctgg catgcgtgcgatc attgatgcgatc ttatatacaca agtggacac 600
gaagaagggtt tgccgcacga aaccgcctta atcaaacacgc ttggccgttga tggcaagtcc 660
gtcatcaatc caccgttacat tccatgttgcatt aatgggggtt ttggccgttgc ttggccgtt 720
gttcaaaaag caccgttgcgatc ttggccgttgc ttggccgttgc ttggccgttgc 780
gttggccgttgc tgaacgggcgatc aatgggttgcgatc aaccgcgttgc tgcgttgc acatgttac 840
atgcgtgcgatc caaaggcatc aggaatggag gtatgttgc 879

SEQ ID NO: 21      moltype = AA length = 101
FEATURE           Location/Qualifiers
source            1..101
                  mol_type = protein
                  organism = Lactobacillus paracasei

SEQUENCE: 21
MEIKHPATAG TLESSDIQIT LSPATSGVAI QLQSSVEKFQ GHQIRSVIEA TLAKLGIENV 60
AVDANDKGAL DCTIKARTIA AVYRASDNKT FDWEEINAWI N 101

SEQ ID NO: 22      moltype = DNA length = 306
FEATURE           Location/Qualifiers
source            1..306
                  mol_type = other DNA
                  organism = Lactobacillus paracasei

SEQUENCE: 22
atggaaatata accatcctgc gactgttgcgatc acgtgttgcgatc tcaaaatcacc 60
ttgtcacccgg ctaccatgttgcgatc ggtgcgcatt caactgcggatc gcaatgttgcgatc aaaaacatgtt 120
ggtcatcaatc ttcgtatgttgcgatc catttgcgttgcgatc accttgcgttgcgatc agttaggttgcgatc cgaaaatgtt 180
ggcggttgcgatc cgaatgttgcgatc agggcgccgttgcgatc gattgttgcgatc tcaaggcgccgatc gacgttgcgatc 240
gtctgtttatc gtgcgttgcgatc caataagatgcgatc tttgttgcgatc aggatgttgcgatc aaccgttgcgatc 300
aattaaatc 306

SEQ ID NO: 23      moltype = AA length = 467
FEATURE           Location/Qualifiers
source            1..467
                  mol_type = protein
                  organism = Lactobacillus paracasei

SEQUENCE: 23
MPKQKVQFME TVLRDGQQL IATRMPMSDI LPILDKMDAA GYASLEMWGG ATFDACLRYL 60
NEDPWERLRLK IRKAVKHTKL QMLLRGQNLQ GYKNYADDVW TDFVTKSVEN GIDIIIRIFDA 120
LNDTRNRLTA LEATKQAGGH AQLAICYTT DFHTIDYFIK LAKDMADMGA DSIAIKDMAG 180
ILTPQKAFLD VIRGIKQEISV PLEVHTHATA GMAEMTYLEA VRAGADIIDT AVSPFAGGTS 240
QPATESMLVA LQDLGYPTDV DLSTVSDIAT YFAPIRDFFR ESGQLNPRVK DVEPKSLIYQ 300
VPGGMLSNNN AQLKDQGQEA LYGDVLKEPV RVRADLGYPV LVTPLSQMVG TQSLMNVMMSG 360
ERYKLIPNEI KDYVRGLYGR PPVIAPEMV KKIIGDAPVW TQRPADLIKP QMPDFRQAIA 420
QYAHDEEDVLSYALFPDQAK DFLGRREDPF YDVPEQKVSL SFEPTHD 467

SEQ ID NO: 24      moltype = DNA length = 1404
FEATURE           Location/Qualifiers
source            1..1404
                  mol_type = other DNA
                  organism = Lactobacillus paracasei

SEQUENCE: 24
atgcctaaac agaaaatgttgcgatc attcatgttgcgatc accgtttgcgatc gtcgttgcgatc acaaaatgttgcgatc 60
atgcgttgcgatc ggtatgttgcgatc cttatgttgcgatc ttggccgttgcgatc tgcgttgcgatc ggatgttgcgatc 120

```

-continued

ggctatcat	ctttggaaat	gtggggcggg	gcaacttttgc	atgcctgtct	ccgttatctg	180
aatgaagatc	cgtggaaacg	gttgcgcgaag	attcgtaagg	cggtcaagca	caccaaatttgc	240
caaataatgtct	tgcgcgggca	aaacttgtta	ggttacaaaa	actatgcgcga	cgatgtggtc	300
actgactttgc	tcacaaatgtc	agtgaaaaac	gggattgata	tcatcgat	tttcgatgcg	360
ttgaatgata	cacgcacatttgc	gcaacggcgc	cttgaagcga	cgaagcaagc	aggcgggcat	420
gttcacatgc	ctatgttgc	cacaaccagt	gatttccata	cgatcgacta	cttcatcaag	480
tttagccaaag	acatggctga	catgggtgcg	gattcaatttgc	aatcaaaa	catggcaggc	540
atttttaacc	cacagaaggc	gtttgatctg	gttaccggta	ttaagcaggaa	aatcagctg	600
ccactgaaag	tgcatacgca	cgccacecg	ggtatggctg	agatgacgt	tctggaaagca	660
gttgcgcgcg	gtgtgtatgc	cattgatacc	cgcggttcgc	catttgcgg	cgccaccagt	720
cagccagcaa	daataatccat	gtgtgtgcg	ttgcaagatcc	ttggatattcc	gactgtgtt	780
gatccaagca	cggtcaagtgc	cateccact	tacttgcgc	cgatccgcga	tcgattccgc	840
gagtcgcgc	aactgaatcc	gcgegtgaaa	gatgttgaa	ctaaatccc	gatctatcc	900
gtgccaggcg	ggatgtgtc	taacatgttgc	gcccactaa	aaatcaagg	acaagaagcg	960
ctttatgtgg	atgtttggaa	ggaagtccgc	cgtgtccgcg	ctgacttag	ctatccgcgc	1020
ttggatcac	cgctgtcgca	gatgtgggc	acacaaatgt	ttgtqaatgt	catgacgggt	1080
gagcgttata	agttgatcc	aaatgaaatt	aggattacg	tgccgcgcct	ttatggtcgg	1140
ccgcccgatgg	caattgcacc	cgaaatgttg	aaaaatgtca	ttggatgtgc	accggttgtc	1200
acacaacatgc	ccgcggattt	aatcaaggcc	caatgcctg	atttccgtca	agcgattgcg	1260
caatatgcgc	acgacgaaga	ggatgttca	agctatgtt	ttgtccccaa	tcaagctaaa	1320
gattttcttg	gcccgcgcga	agatccgtt	tatgtatgtc	cgagcagaaa	ggtgtcgcta	1380
agttttgagc	cgacgcacat	ttgaa				1404

SEQ ID NO: 25 moltype = AA length = 374

FEATURE Location/Qualifiers
source 1..374
mol_type = protein
organism = Lactobacillus paracasei

SEQUENCE: 25

MEALIHGITT	ITLGQIAMI	MLIGALLMYLGI	KKEYEPTLLV	PMGLGAILVN	FPGTGVLTVQV	60
VGGTKAEGVL	DVLFKAGINT	ELFPLLIFIG	IGAMIDFGPL	LQNPFMLLF	AAAQFGIFAT	120
VFVAVFFGFN	IKEAASIGII	GAADGPTSIF	VSNQLAPNL	IL	GAITVAAYSY	180
AIKAVITKHE	RRIRMTYKAE	GVSKTTKIL	PIIITIIAGF	IAPISLPLVG	FLMFGNLRLRE	240
CGVLDRLSNT	AQNELVNIVS	ILLGLTISVK	LQADQFLNIQ	TLMIIAFGLF	AFIMDSVGGV	300
LFAKLLNLNFR	KDKINPMIGA	AGISAFPMSS	RVIQKMATDE	DPQNFVLMYA	VGANVSGQIG	360
SVIAGGLLSS	FFGA					374

SEQ ID NO: 26 moltype = DNA length = 1125

FEATURE Location/Qualifiers
source 1..1125
mol_type = other DNA
organism = Lactobacillus paracasei

SEQUENCE: 26

atggaaacgc	tcattcacgg	aatcaccacg	atcacattag	gtcaaatcgc	catgtatgt	60
atccggcgc	tcctgtatgc	tctggaaatc	aaaaaaatgt	atgaaccaac	ccttttagtt	120
cccatgggc	tggggccat	tctgttcaac	tttccaggaa	caggcgtt	gacccaaatgt	180
gttggcgc	ccaaacgcg	aggggtgttgc	gtatgtttat	tcaaaaggccg	tatcaataacg	240
gaactgttcc	cactgttcaat	tttcatcggg	atccggccca	tgtatgtt	ttggaccgtt	300
ttacaaaacc	cattttatgtc	actgttcgtt	gcacgcac	agttccggat	ctttgcacc	360
gtttttgttgc	ctgttttctt	cggttcaat	atcaaagaag	cggttcaat	ttggatcatc	420
gtgtccgcac	acggccgcac	ttccgttac	gttgcgttac	aacttgcgc	aaatctgtt	480
ggggccatca	cagtgcgtc	gtattcgat	atggcattgg	tgccgtat	ccagccatg	540
gccatcaacgg	cagtgcgttac	aaacatgtaa	cgccggatttgc	ggatgtactt	taaggcagaa	600
ggcggttca	agacgcacaaa	aattctgtt	ccatcatta	tcacgttatt	tgccgggttt	660
attggcccg	tttccttacc	gttagtggg	ttccgtatgt	ttggtaactt	gtgtcgagaa	720
tgtgggggtgc	tcgtatgttgc	gtctaaaccc	gcccacaaatgt	aaatgttca	tattgtgtcg	780
attctgttgc	ggcttaacat	ttccgttac	tttgcgttac	atcaatctt	gaacatttca	840
acgttgcgttgc	tcattgttttgc	ttggattttc	gccttcatca	ttggactctgt	cggggggtgt	900
ttgttgcgttgc	aattttgttgc	ttccgttac	aaagataaga	ttaacccat	gtatggggcg	960
ggccgcattt	ccgttttcc	aatgtcgac	cgatgttac	aaaaatgtgc	aaccgtatgt	1020
gatccacaga	attttgttttgc	gtatgttgc	tttgcgttac	atgtttccgg	ccaaatcggt	1080
tctgttgcgttgc	ccggcgactt	gttactatca	ttctttggcc	cataaa		1125

SEQ ID NO: 27 moltype = AA length = 382

FEATURE Location/Qualifiers
source 1..382
mol_type = protein
organism = Escherichia coli

SEQUENCE: 27

MKALHFGAGN	IGRGFIGKLL	ADAGIQLTFA	DVNQVVL DAL	NARHSYQVHV	VGETEQVDTV	60
SGVNAVSSIG	DDVVVDLIAQV	DLVTTAVGPV	VLERIAPAI	KQQVKRKEQG	NESPLNIIAC	120
ENMVRGTTQL	KGHVMNALPE	DAKAWVEEHV	GFVDSAVDRI	VPPSASATND	PLEVTVETFS	180
EWIVDKTQFK	GALPNIPGME	LTDNLMAFVE	RKLFTLN	GHQTIRDA	L	240
DEKIRAVVKG	AMEESGAVLI	KRYGFDADKH	AAVIQKILGR	FENPYLKDDV	ERVGRQPLRK	300
LSAGDRLIKP	LLGTLEYGLP	HKNLIEGIAA	AMHFRSEDDP	QAQELAALIA	DKGPQAALAQ	360
ISGLDANSEV	VSEAVTAYKA	MQ				382

-continued

```

SEQ ID NO: 28      moltype = DNA  length = 1149
FEATURE
misc_feature
1..1149
note = Encoding SEQ ID NO: 27 and codon-optimized for
expression in Saccharomyces cerevisiae
source
1..1149
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 28
atgaaggcac tgcacttcgg ggctggcaac ataggctcg gctttatagg caagttacta 60
gtctgacccg gtatccaact aaccttgcgatgtaaatc aggttgtctt agacgccttg 120
aatgcaaggc atagttatca agtccatgtatggcgaa cggAACAGGT tgatacggtg 180
tccggagtga atgcagtgc ttctataggc gatgcacgtgg tcgtatcgat tgccacaagg 240
gacttggta ccaactgcgggt aggaccatgc gtcttagaaatc gtatagctcc tgcaatcgcc 300
aagggtcagg tcaagaggaa ggacggccggc aacgagaccc cctgtatattt cattgttgc 360
gaaaacatgg ttagggggac cactcagttg aaaggccacg taatgaacgc attgccagag 420
gtatgcgaaagg ctctggataga agacatgtc gttttgtcg attcagctgt tgacagaatc 480
gtgcggccgtt ccgcgttgc tactaacgc cccgttgggg tcacagtata aacttccgc 540
gaatgtatcg tagacaaaac acaattttaaatc ggcccccgtc ctaacatacc gggatggaa 600
ctaacagaca atttaatggc attcgtggag agaaaatttat ttactcttaa cacaggccat 660
gccccatccgg cttatattgg gaaatgttgcg gcccatcaga ctataagaga tgccattcta 720
gacgaaaaaaa tccgtgcgtt cgttgcgttgcgaaatggccgc cgtccgttgc 780
aagcgttacg gttttgcgatgc agataatgcgatgtc ggcgcgtata ttccggaaatc ccttggccgt 840
ttcgaaaatc catatttgcgaa ggacgtgtg gggcgtgtgg gtcgtcggcc gttggggaa 900
ttatctgtgtg gtggccgtct aattaatgcgatgtc gtcgttgcgaa ctttggatgc cggactgcca 960
cataagaaacc ttagatgggg gatgttgcgttgcgatgtc gcaatgttgcgatgtc agatgttgc 1020
caggcacaag agttggctgc tctgttgcgaa gacaatggcc ctcggccgc tttggccgc 1080
atctcggcc tagatgttgcgaa tagatgttgcgatgtc gtcgttgcgatgtc gtcgttgcgatgtc 1140
atgtcaataa 1149

SEQ ID NO: 29      moltype = AA  length = 259
FEATURE
source
1..259
mol_type = protein
organism = Escherichia coli

SEQUENCE: 29
MNQVAVVIVGG GQTLGAFFLCH GLAAEGYRVA VVDIQSDKAA NVAQEINAEY GESMAYGFGA 60
DATSEQSVLIA LSRGVDEIFG RVDLLVYSAG IAKAAFISDF QLGDFDRSLQ VNLVGYFLCA 120
REFSRLMIRD GIQQGRRIQIN SKSGKVGSKH NSGYSAAKFG GVGLTQSLAL DLAEYGITVH 180
SMLMGNLKPS PMFQSLLPQY ATKLGIKPDQ VEQYYIDKVP LKRGCDYQDV LNMLLFYASP 240
KASYCTGQSI NVTGGQVMF 259

SEQ ID NO: 30      moltype = DNA  length = 780
FEATURE
misc_feature
1..780
note = Encoding SEQ ID NO: 29 and codon-optimized for
expression in Saccharomyces cerevisiae
source
1..780
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 30
atgaaccagg tggcgttgcgtt gatcgccggc ggccagacac ttggagcggtt cctttgttac 60
ggctttagcgg cccgggggttta cagggttagcc gtcgttagaca ttcaatgttgc taaaggcgttgc 120
aacgtcgccatc aagatataatc tggggatataatc gggggatgttgc tggccctacgg attttgggtgt 180
gtatgcacta gcaacacgtc ttgttgcgttgc ttccatgggg gggtagatgttgc aattttccgg 240
cgtgttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 300
caatttaggtt attttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 360
aggggatgtt ccacacttgc gatgttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 420
agtaatgttgc gatgttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 480
gggtgttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 540
tctctgtgtc tggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 600
gcccacggaaac tggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 660
ttggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 720
aaggcgttgcgttgc ttccatgggg gggggatgttgc ttccatgggg gggggatgttgc ttccatgggg gggtagatgttgc aattttccgg 780

SEQ ID NO: 31      moltype = AA  length = 266
FEATURE
source
1..266
mol_type = protein
organism = Lactobacillus paracasei

SEQUENCE: 31
MSDWLGLDGK TIVVTGGSSG IGAIAVKELI NNGATVVNGD LKEGDFKDPN LKYVHTDVD 60
PDEVENLAAT AEKINGEIWG VVNNAGINKP RVLVDPKDPH GKYLEDVKTF EQIPSVNKS 120
PFLVVSQAVVR RMVKQGHGVV VNMSSEAGLE GSVGQSVYSA SKGAINGFTR SWAKELGKYN 180
IRVVGVAPGI MEATGLRTPS YEEALAYTRD TTVDAIRAGY SSTSTTPLGR SGKLSEVGDL 240

```

-continued

VNYFLSNRAS YITGVTTNVA GGKSRG	266
SEQ ID NO: 32	moltype = DNA length = 801
FEATURE	Location/Qualifiers
misc_feature	1..801
	note = Encoding SEQ ID NO: 31 and codon-optimized for expression in <i>Saccharomyces cerevisiae</i>
source	1..801
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 32	
atgagtgatt ggcttggct ggatggcaaa accatcgtc tcactgggg ttccctctggc 60	
atccggcgg ctatcgtaa agaattgatt aataacggcg cgactgttgt taacggcgc 120	
ttgaaaagaag gggactcaa agatccgaat cttaaagtgt ttcacacggg cgttacagat 180	
cccgatggat ttgagaacct tgctcaacc gctgaaaaaa caaatggca aatttgggt 240	
gttggtaaca acgctggcat caacaaggct cgtgtttgg tagatcttaa ggaccacat 300	
ggcaagtatg aattggatgt gaagacattt gaacagattt ttcctcgtaa cgttaatca 360	
gttttccttgg ttcccaggc cgtggccgc cgtatgggtt agcaaggcca tggcggtt 420	
gttaacatgt catccgaagc aggttggtag ggcagcgttg gtcaatcgat gtactcagca 480	
tcaaaggccg caatcaatgg ctttacacggg tcttgggcta aggaacttgg taaatacac 540	
attcgctgc ttggcgctgc tccaggatc atgaaagctt cgggcttgcg tacggcaagc 600	
taugaagaag ctttggctt caccggat actaccgtt acgcttatttt tgctggctac 660	
tcaagttacca gcaccatcc ttcggctgc tcaggttaatgt tgctctgaat tggggactg 720	
gtttaattttt ttctatctaaa ccgtgccttc tatattactg gcgttacaaat taatgttgt 780	
ggcgggaaat ctctggctta a 801	
SEQ ID NO: 33	moltype = AA length = 189
FEATURE	Location/Qualifiers
source	1..189
	mol_type = protein
	organism = <i>Lactobacillus paracasei</i>
SEQUENCE: 33	
MQYVSDFAAG FMKLFQFQGGK TFISWMTSIV PVVLLLVLV NTIIAFIGEE RIERFAQKAS 60	
RNVLMRLYLVLP PFLAAFPMLGN PMCFTLARFL PEYYKPSYAA AQAQFCHTSN GVFPHINPGE 120	
LFVWLGLIAQG VQKLGLNQMD LAIRYMLVGI VMNFIGGWVT DFTTAYVSKQ TGITLSKTV 180	
LSARNGQEAA 189	
SEQ ID NO: 34	moltype = DNA length = 570
FEATURE	Location/Qualifiers
misc_feature	1..570
	note = Encoding SEQ ID NO: 33 and codon-optimized to prevent homologous recombination with native host gene
source	1..570
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 34	
atgcagttt tttctgtactt tgcagcggc ttcatgaaat tggccggac cggggggcaag 60	
acttttatcat catggatgac ttccatcgcc ccagggttgc ttgtgtctt ggttttggat 120	
aacactatttccat tccgttttcat cggcggaaat cggatcgaaat gtttttgcaca aaaggcttgc 180	
cgtaatgtcc ttatgtcggtt cctgggttta ccatttttgg cagtttccat gctgggtat 240	
ccgatgttttgc taccatgttgc tcggcccttccat ccagaatact ataaggccat ctattacgcc 300	
gctcaagcccc aatttttgcac caccttcaac ggcgttttgc cgcacattaa tccctggcga 360	
ctgttgcgtt ggctgggtat tgctcaaggat gtcacaaat tggggcttgc ccaaatggat 420	
ttggccatcc ggtatgtt agttggcattt gttatgttactt tcatcggttgg ttgggttacc 480	
gacttttacaa ctgcatatgtt ttcaagaaat acgggtatca ccttgcctaa aactgtggat 540	
ttatccgcac gtaatggtca ggaagctt 570	
SEQ ID NO: 35	moltype = AA length = 372
FEATURE	Location/Qualifiers
source	1..372
	mol_type = protein
	organism = <i>Lactobacillus paracasei</i>
SEQUENCE: 35	
MADQKWHSIQ VVKGSGGYGG PLTITPTEQK HKFIYVTGNN RPAIVDKIVE LTGMEAVDGF 60	
KTSIPEDETA VAIIDCGGTL RCGIYPKKNI LTINVLPTGK SGPLAKYIVP KLYVSNVDVN 120	
QITALPDDAV PDQSLNGVPF DQRGEAGKOH AALAESAAQK ATATEAKTTA AKDQEAEEAR 180	
ETKFDTNKTI TAQMKKPNFI ARIGIGAGKV IATFNQAAKD SVQTMNLNTVI PFMAFVALLI 240	
GIIQGSGLGS WFAKLMTPLA GNFGGLIVIG FICSLPFLSP ILGPVGAVIAQ VIGTLIGVEI 300	
GRGNIQPOYA LPALFAINTQ NAADFIPVGL GLEEDADSKTV EVGVVSVLYS RFLNGVPRVV 360	
VAWLASFGLY AK 372	
SEQ ID NO: 36	moltype = DNA length = 1119
FEATURE	Location/Qualifiers
misc_feature	1..1119
	note = Encoding SEQ ID NO: 35 and codon-optimized to prevent homologous recombination with the native host gene

-continued

```

source          1..1119
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 36
atggctgatc aaaagtggca ctccattcaa gtggtcaagg gtagcggtgg ttacggtggt 60
ccattgacca tcactccaac tgaacagaag cacaaggttt ttacgtgac gggggtaat 120
cgtccctgaga ttgttgacaa gatcggttgc ctgaccggta tggaaagctgt ggatggttc 180
aaaactctta ttccagaaga tgaaacggct gtgcattttt tcgactcgcc cggtacccctg 240
cggtgcggta ttacccaaa aaagaacatt cttacgatta acgttcccg gactggtaag 300
tcggccctc ttgtcaagta tatecggttccg aagtgtatg tggccaaacgt tgatgttaat 360
cagattaccg ttcttaccaaa ccatgttcccg ccagaccaat cattgaacgg tgccccc 420
gatcagcggg gtgaagctgg caaaaacac gcccgttgc cagaaatgc tgctcccaa 480
gctaccgcta ccgaggccaa aaccactgtc gccaaggatc aggaagctgc tgaagctcgc 540
gaaaacgaaat ttggacccaa caagacaatc accgcacaaa tgaagaaacc taacttcat 600
gcacgcgtcg tggcaggatc atggcaaccc tcaaccaacg ggtaaaagac 660
ttctgtccaaa ccattgttgc caccgttattt ccattttatgg ctttgttgc ttctttatc 720
ggtttatcc aggggtcagg tctgggtca tgggttgcata aactttagac tccattagcc 780
ggcaacgttat tccgggttat tggatcggtt ttcatgttgc ctttgcattt cttgttccca 840
atccctcgcc cgggggtgtt tattgcacaa gtcattgttgc ccattgttgc cgttgagatc 900
ggtcgttgcata catccaaacc gcaatacgctt acccgttgc ttttttgcata taacaccgc 960
aatgcgcgtg attttatcc tgggttgcata ggggttgcata aagcggatc caagccgtt 1020
gaagtgcgttgc tggtagtgc tttgtactcc cgtttccgtt atggcggttcc acgcgttgc 1080
gtcgcttgcata cggccgttgc gccaacttgc 1119

SEQ ID NO: 37      moltype = AA length = 126
FEATURE           Location/Qualifiers
source            1..126
               mol_type = protein
               organism = Lactobacillus paracasei

SEQUENCE: 37
MSSLAETVKY ETKILEVGS ARGFKDINMA ILFGDEAPDA LRSSCFIINV NKILEPIEVG 60
DVMTFDDQSY KITAVGNEVN TNGLNLGHTA IVPDGSTTPE LAGSLYLEEK TYPELDVGTT 120
IKIIRA

SEQ ID NO: 38      moltype = DNA length = 381
FEATURE           Location/Qualifiers
misc_feature      1..381
note = Encoding SEQ ID NO: 37 and codon-optimized to
               prevent homologous recombination with native host gene
source            1..381
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 38
atgtcgcttgc tggctgagac cgttaataac gaaacaaaga ttttggaaat tggcagcgaa 60
gctcgcggct tcaaaatcat caatatggca attctgttgc gtgtatgaa tccggacgcc 120
cttcgttgc tcatgttcat catcaacgtt aataagattc tggaaaccaat tgaagttggg 180
gtatgtcatgat cttttgtatc tcaaaatgcata aaaaatcaccc ctgttgcacaa tgaagttaac 240
actaaacttgg gtaacccggg ccataccgtt atcggttttgc acgggttccac gactccggaa 300
ttggcggggctt cttgtactt ggaagaaatg acgttatccat aactcgatgt tggcactacc 360
atcaaaaatca ttccggcttgc a 381

SEQ ID NO: 39      moltype = AA length = 384
FEATURE           Location/Qualifiers
source            1..384
               mol_type = protein
               organism = Lactobacillus paracasei

SEQUENCE: 39
MMEA VHFAG NIGR GFIGET LAANGKINF VDVNETIINA LNQRGEYTIT LAAPGEKKIH 60
VDNVDGLNNA KDPEAVVKAA AQADLVTTAI GPKILPPIAP LIAQGLQARD AANNHQALDV 120
IAECAENMIGQS QSLKKS VYEH LDDAGKTFAD TYVGF PNAAV DRIVPQQKH DPLAVSVEDF 180
KEWVVDQESQM KNKDLKLKTV DYVPDLEPYI ERKLFSVN TGG HATTAYTGKY LGYTTIGDAI 240
KDPKVF NQAK GALAETRSLL LSEFKNFDEK DLEN YQNRL QRFQNPYISD DISRVARTPI 300
RKLG YDERFI RPIRELK EGN LNYSVLMDTV GMMFHYVEPN DAEAVKLQAM LKDQPLVDVI 360
KEVTGLKDAG LIDEVEASVK SKDR 384

SEQ ID NO: 40      moltype = DNA length = 1155
FEATURE           Location/Qualifiers
misc_feature      1..1155
note = Encoding SEQ ID NO: 39 and codon-optimized to
               prevent homologous recombination with native host gene
source            1..1155
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 40
atgatggaaatg cagttcat tgggtcaggat aacatcgcc ggggttcat tggtaaaaca 60
ttggccgcttcaaa aatataatttc gtcgacgttgc atgaaacgtt tatcaatgtt 120

```

-continued

ttaaacccagc	gcggcgaata	cactatca	ctcgccggcac	ctggcgaaaa	gaagattcac	180
gttgcaca	tcggacggct	gaataacccg	aaagaccgg	aagcagtct	taaggcaatt	240
gcccaga	ctgtttgt	gacccatc	ggcccaaaga	tttcccaat	tatcgaccca	300
cttattgtc	aaaggttgc	agcacgtat	gctgcgaaca	accatcaagc	cttgacgtg	360
atcgcttgc	aaaacatgtat	cgaggctca	caatcccta	agaagagtgt	ctatgaacat	420
ttggatgtat	ctggtaaaac	cttcggcgat	acccatgtcg	gtttccctaa	tgccgctgtg	480
gatcgat	tcccgcaaca	aaagcatgtat	gaccactatc	cggtcagtgt	tgaagatttc	540
aagaatggg	ttgtcgatga	aagtcaatg	aaagataaagg	attnaaagg	gaagactgtt	600
gactacgtt	ctgaccccg	gccttacatt	aaacgtaaatgt	tggttccgt	taatactgg	660
catgcaacca	ctgcgtat	aggtaataac	cttggctca	caacgtatgg	tgacccat	720
aaggatcct	aggttttaa	ccaaaggc	gggtcgctgg	ccgaaacacg	tagtctgtt	780
cttcagaat	tcaaaaactt	tgatgaaaaa	gacttggaaa	actacaaaaa	ccgcgtt	840
caacggttt	aaaaccata	tatctccgac	gacatctca	gtgttcccc	gaccctatt	900
cgcaagtgg	gttatgtga	acgttccatc	cgcccaattt	gtgagctgaa	ggaacgtggc	960
ttaaattact	cagttctgtat	ggataccgtt	ggtagatgtat	tecatatgt	tgaaccaa	1020
gtatggcga	caggtaaagct	tcaaggat	tttggggatc	aaccgttgg	ggacgtt	1080
aaggaaagtt	caggctgaa	ggacgctggc	cttattgtat	aaatggggc	ctcagttaaa	1140
tcaaaggacc	gtttaa					1155

SEQ ID NO: 41 moltype = AA length = 608

FEATURE Location/Qualifiers

source 1..608

mol_type = protein

organism = Lactobacillus paracasei

SEQUENCE: 41

MGAKTANTPA	AEKKKPNLKA	GMQSFGTKLS	GMVLPNIGAF	IAWGLITAIF	LKGGWYPNAQ	60
LAKMISPMVT	YLLPLLIASF	GGSMVAGHRG	GVVGAIAMG	VIVGTDVPMF	IGAMVMGPLG	120
GWCICKWVDR	FQDKIKQGF	MLVNNFSAGI	IGMLLAIVGF	FLMGPPISTL	TNGMATGVDW	180
IINHGLLWVA	NVFIEPAKIL	FLNNNAINQGI	LTPLGQAA	EHGKSILFL	EPDPGPGGLGV	240
LLAFAFLFGKG	SAKGAPSAPAI	IIFHLGGIHE	IYPPYVLMKP	ALFLSVMAGG	VTGTTLFISIF	300
NVGLKSSPSP	GSIFALFAM	PVNIGNYIGL	IVGVTGATLV	SFLISAVILR	RDKSASGDEL	360
AEEAKMKS	KAEAKGQONV	AAAKDVMSSA	KG1KQIIIPAC	DAGMGSSAMG	ASILEDKVKK	420
AGLDLSVTNT	AISNLQDKPG	LLVVTQEELA	DRAKDKTPDA	AHIAVDNFLN	SPKYDEIIAS	480
LKAEAVGGTD	EAMPATETSK	AKQETPEDEL	KELLDLKITE	VDFLHHHDQNI	GSATMAQATF	540
RAELRKLNKD	VKVRNVAIGE	IDDKDNVLII	ASKETARRVK	LQFANQVYVT	VDGLLNATNY	600
DKLIEKMK						608

SEQ ID NO: 42 moltype = DNA length = 1827

FEATURE Location/Qualifiers

misc_feature 1..1827

note = Encoding SEQ ID NO: 41 and codon-optimized to prevent homologous recombination with native host gene

source 1..1827

mol_type = other DNA

organism = synthetic construct

SEQUENCE: 42

atgggtcaaa	agacggcaaa	tactccagca	gcagaaaaaga	agaagttcaa	cctgaaggcc	60
gttatgc	aaa	ggttcgtac	caagtttct	ggtatggttc	ttcctaacat	120
atcgcttgg	ggttgc	atcac	agctatctt	ctcaaggccg	gctggatcc	180
ttggccaaga	tgat	ttcc	tatggttacc	tacttgc	taatgccag	240
gttgttca	ttgttgc	ccat	gttgttgcgtt	gtgtgcgtt	gtgcatatgc	300
gttata	tcgttgc	gtac	gttgttgcgtt	gttgttgcgtt	gtccatgggt	360
ggtgttgc	tca	aa	gttgttgcgtt	gttgttgcgtt	tcctttgggc	420
atgtgttgc	aca	actt	cgcagg	atggcatgc	ttccaggata	480
ttctgttgc	gg	ccat	atggcatgc	atggcatgc	agat	540
attatca	at	ggc	atca	atggcatgc	atggcatgc	600
ttctgttgc	acc	cc	tcagg	gttgttgcgtt	tttgcatttgc	660
gaacatgtt	at	tttgcatttgc	gttgttgcgtt	gttgttgcgtt	ggcagg	720
tttgttgc	tc	tc	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	780
atcatttc	t	t	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	840
gcottgtt	t	t	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	900
aatgttgc	taaa	atc	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	960
ccggtaata	ttgg	at	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1020
tcttc	ttgt	at	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1080
gccc	aggat	aa	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1140
gcac	ggcc	ca	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1200
gacg	ctgg	ca	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1260
gctgg	ggct	ca	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1320
cttt	gggt	ta	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1380
gctcat	atttgc	ca	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1440
tttgc	agggtt	cc	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1500
gcaaa	ggca	aa	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1560
gttgactt	ttcacc	at	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1620
cgtgc	ccat	aa	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1680
tttgc	aggat	aa	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1740
tttgc	aggat	tt	atcgttgcgtt	atcgttgcgtt	atcgttgcgtt	1800

-continued

```

gacaaaattga tcgagaagat gaaataa 1827
SEQ ID NO: 43 moltype = AA length = 158
FEATURE Location/Qualifiers
source 1..158
mol_type = protein
organism = Lactobacillus paracasei
SEQUENCE: 43
MKSKKKLIEGD MMKGLDVKTI KLGQEAKTKE EAIRQAGQLL VDNGNVEPAY IDSMLDRNRD 60
VSVYMGNFIA IPHGTEAGMK YIKSTAISIV QYPWGVWDSD DPADENLVTV VFGIAGLNGE 120
HILKLLSQIAL YCSDVENVQK LADAQTPEEI VNLLKEVE 158

SEQ ID NO: 44 moltype = DNA length = 477
FEATURE Location/Qualifiers
misc_feature 1..477
note = Encoding SEQ ID NO: 43 and codon-optimized to
prevent homologous recombination with native host gene
source 1..477
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 44
atgaaatcta aagaaggatcg cgaagggtac atgatgaaag gattggatgt taaaacgatc 60
aaaccttggcc aagaaggccaa aacgaaaggcc gaagcttatcc gtcaggatgg ccaattgtctc 120
gttgataacg gtaatgtga accaggctt attgatctca ttgtggaccc taacccgcac 180
gttagtgttt acatggccaa ttccattgtt attccacatg ggacagaagc ttgtatgaag 240
tacattaaaga gtacggctat ttctatcgat cagtaccggt ggggcgtgga ctggtcagac 300
gaccggctg atgagaactt agttactgtt gtttccggta ttgcccgtt gaacgggtgaa 360
cacctgaagc tgctgttca gatcgccatttattgtcccg atgttggaaa cgttcaaaag 420
ttggcagatg ctcagacgccc agaagaaatc gtcaacttgt taaaggaaatc tgaataa 477

SEQ ID NO: 45 moltype = AA length = 360
FEATURE Location/Qualifiers
source 1..360
mol_type = protein
organism = Entamoeba nuttalli
SEQUENCE: 45
MKGLAMLGIG RIGWIEKKIP ECGPLDALVR PLALAPCTSD THTVWAGAIG DRHDMILGHE 60
AVGQIVKVGS LVKRLKVGDK VIVPAITPDW GEEESQRGVP MHSGGMLGGW KFSNPKDGVF 120
SEVFHVNEAD ANLALLPRDI KPEDAVMLSD MVTTFGHGAE LANIKLGDTV CVIGIGPVGL 180
MSVAGANHG AGRIFAVGSR HKCCDIALEY GATDIINYKN GDIVEQILKA TDGKGVDKVV 240
IAGGDVHTFA QAVKMIKPGS DIGNVNYLGE GDNDIDIPRSE WGVGMGHKHI HGGLTPGGRV 300
RMEKLASLIS TGKLDTSKLI THRFEGLEKV EDALMLMKNK PADLIKPVVR IHYDDDETLH 360

SEQ ID NO: 46 moltype = DNA length = 1083
FEATURE Location/Qualifiers
misc_feature 1..1083
note = Encoding SEQ ID NO: 45 and codon-optimized for
expression in Saccharomyces cerevisiae
source 1..1083
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 46
atgaagggtt tggctatgtt gggatcggtt acaaactcggtt ggatcgaaaa gaagatccca 60
aatgtggc cattggacgc tttggtaga ccattggctt tggctccatg tacttctgac 120
acttcacactg tttggctgg tgcgtatcggtt gacagacacg acatgtatcc gggtcacgaa 180
gctgtggc aaatcgatccaa ggttggatcc ttggatccaa gatttggatgg tggatccaa 240
gttatecgatc cagctatcac tccagactgg ggttggatgg aatctcaaa aggttaccca 300
atgcactctg gtggatgtt ggggtggatgg aatgttctcta acttcaagga cgggttttc 360
tctgaagt tccacgttac cgaacgttac gctaacttgc ctttggttcc aagagacatc 420
aagccagaag acgtctgttat gttgtctgac atggttacta ctgggttccaa cgggtgtccaa 480
ttggctaaaca tcaaggatggg tgcactatgtt tggatccatcg tgcgtatcc acgttggatgg 540
atgtctgttg ctggatccaa ccacttgggtt gctggatggaa tcttgcgtgt tggatccaa 600
aagcaactgtt gtgacatcgc tttggatatac ggttggatccatcg acatcatccaa ctacaagaac 660
gggtgacatcg ttggatccaaat ctggatccaaat ctggatccatcg acgttggatgg 720
atcgctggatcg tgcacgttca cacttgcgtt caagctgttca agatgtatccaa gccaggatcc 780
gacatcgatccaa acgttacta ctgggttccaa ggttggatccaa tgcacatccc aagatctgaa 840
tgggggtgtt gatgggttca caagoacatc cacgttggatgg tgcgtatcc acgttggatgg 900
agaatggaaa agttggatccaaat ttgtatccatcg actggatccatcg tggatccatcg 960
acttcacatcg tggatccaaat ctggatccatcg acgttggatgg tgcgtatcc acgttggatgg 1020
ccagctgttca gatggatccaaat ctggatccatcg acgttggatgg tgcgtatcc acgttggatgg 1080
taa 1083

SEQ ID NO: 47 moltype = AA length = 910
FEATURE Location/Qualifiers
source 1..910
mol_type = protein

```

-continued

organism = Bifidobacterium adolescentis	
SEQUENCE: 47	
MADAKKKEEP TKPTPEEKLA AAAAEVDALV KKGLKALDEF EKLDQKQVDH IAVAKASVAAL	60
NKHLVLAKMA VEETHRGGLVE DKATKNIFAC EHVTNYLAGQ KTVGIIREDL VLGIDEIAEP	120
VGVVAGVTPV TNPTSTAIFK SLIALKTRCP IIFGFHPGAQ NCSVAAKIV RDAAAIAGAP	180
ENCIQWIEHNG SIEATGALMK HDGVATILLAT GGPGMVKAAY SSGKPALGVG AGNAPAVVDK	240
NNDVVRRAAND LILSKHFDYQ MICATEQAII ADKDIYAPLV KELKRRKAYF VNADEKEAKLE	300
QYMFCTAYS GQTPLNSVVG PKGSPQVIYK AAGFPIPEDA TILAEECKEV GENEPLTMEK	360
LAPVQAVLKS DNKEQAFEMC EAMLKHGAGH TAAIHTNDRD LVREYQGRMH ACRIIWNSPS	420
SLGGVQDIYN AIAPSLLTLCG GSYYGGSVSG NVQAVNLINI KRIARRNNNM QWFKIPAKTY	480
FEPNNAIKYLE DMYGIEKAVI CKDVKVMQELG DVDTKIIDQLR ARSNRVTFR IDYVEPEPSV	540
ETVERGAAMM REEFEPDTII AVGGGSPMDA SKIMMLLYEH PEISFSVRE FKFDIRKRAF	600
KIPPLGKKAK LVC IPTSSGT GSEVTPFAVI TDHKTYGYKYP ITDYALTTPSV AIVDPVLART	660
QPRKLASDAG FDALTHAFEA YVSVYANDFT DGMALHAALK VWDNLAEHSVN GEPGEKTRA	720
QEKMHNAAAT AGMAGFSALP GMCHGMAHTI GALCHVAGHR TNSILLPYVI RYNGSVEPEP	780
TSPWKSYNKYI APERYQETAK NLGVNPCKTP EEGVENLAKA VEDYRDNKLG MNKSFQRCGV	840
DEDYYWSIID QIGMRAYEDQ CAPANPRIPO IEDMKDIAIA AYYGVSQAEQ HKLRLVRQROGE	900
AATEEASERA	910
SEQ ID NO: 48	molttype = DNA length = 2733
FEATURE	Location/Qualifiers
misc_feature	1..2733
	note = Encoding SEQ ID NO: 47 and codon-optimized for expression in <i>Saccharomyces cerevisiae</i>
source	1..2733
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 48	
atggccgacg ccaagaagaa agaagaacct actaaggcca ccccagaaga aaaattggct	60
gctgtcttgc ctgaaggta tgctttgggt aagaaaggtt tgaaggccctt ggacgaattc	120
gaaaatttgat atccaaagcga agtcgcatac acgttgtcta aagcttcagt tgctgttttg	180
aacaaaccatt tggttttgtc taatgtggcc gttqaaggaa ctcatagagg ttgtgtttggaa	240
gataaggccca ccaagaatata tttcgcttttggaa gaaatgtca ccaacttattt ggctgttcaa	300
aagaccgttg gtatcattag agaagatgtat gttttgggtt tcgacgaaat tgctgaacca	360
gttgggttgtt ttgttgttgc tactccatg actaatccaa cttctacccg tattttcaag	420
tcctgttatttgc cttgttttgtc aagatgttccca attatcttg ttgttcatcc aggtgttcaa	480
aactgttctg ttgttgttgc taaaatcggtt agagatgtctt agatgttccca ttatgttgc tggtgttccca	540
gaaaactgttata ttcaatggat tgaacaccca tccatttgaa ctactgttgc ttgtgttgc	600
cacgtgttgc ttgttactat ttttgcgttactt ggtgttccatg gtatgtttaa ggctgttcat	660
ttttttgttgc aaccagctttt ggggttttgtt gctgttataatg ctccaggatata ttgtgttgc	720
aacgtgtatgtt tggttagagc tgccaaacatg ttgtttttttt ctaagactt cgactacgtt	780
atgatttgc ttactgttgc agtatttata gccgataagg atatctatgc tccattttgtc	840
aaagaattgtt agagaagaaa ggcctacttc gttatgttgc acgaaaaaaacg taagtgttggaa	900
cagtatgtatgtt tggttgttgc ctgttgcgtt ggttcaactc caaagtgttgc ttctgtttttt	960
ccaggatgtt ccccaacatgtt tattgttgc gttccgggtt tgcgaaatccca aaagatgtct	1020
acaattttggc ccgtgtatgtt taaagaagtc ggagaaaaacg aaccattgtac catggaaaaaa	1080
ttggcaccatgttcaacatgtt ttgttagttcc gataacaaag aacaaggccctt cgaaatgttgc	1140
gaaggatgtatgtt gtaaacatgtt tggttgttgc atgtgttgc ttcatacaaa cgatagagac	1200
ttgtgttgttgc aatacggttca aagaatgtatgtt gccttcggatata ttatgggttgc ctcttcacatct	1260
tcttttgttgc tggttgttgc tatctacaatgtt gctattgttgc catctttgtc ttgtgttgttgc	1320
ggttcttgc tggttgttgc ttgttgttgc aatgttgc tttttttttt gatgttgc ttgtgttgttgc	1380
aagagaatgtt cttagaaatgtt caacaacatgtt caatgttgc agatgttgc taaagatcttac	1440
ttgtgttgttgc aacccatcaatgtt gtcgttgc aatgttgc tttttttttt gatgttgc ttgtgttgttgc	1500
gttttgttgc gtttgcgttgc atgtgttgc ttcatacaaa cgatagagac	1560
gccagatcttca acagaggatccatc ttccatgttgc atcgattacg ttgttgttgc accatcttgc	1620
gaaacatgtt aaagggtttgtt gtcgttgc atgtgttgc ttgttgttgc tttttttttt gatgttgc	1680
gttgggttgttgc ttgttgttgc aatgttgc tttttttttt gatgttgc ttgttgttgc	1740
ccagaaatttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	1800
aaagatttttttttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	1860
tttttgttgc ttgttgttgc aatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc	1920
attaccatgttgc atgtgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc	1980
caacatgttgc aatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2040
tacgtttctgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2100
tttttgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2160
tttttgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2220
tttttgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2280
actaacttccatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2340
acatcttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2400
aaagatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2460
aaagatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2520
gacgaaatgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2580
tgtgttgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2640
gcttactacgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2700
gctgttgc tttttttttt gatgttgc ttgttgttgc tttttttttt gatgttgc ttgttgttgc	2733

SEQ ID NO: 49 moltype = AA length = 683

-continued

FEATURE	Location/Qualifiers	
source	1..683	
	mol_type = protein	
	organism = <i>Saccharomyces cerevisiae</i>	
SEQUENCE: 49		
MTIKEHKVY EAHNVKALKA PQHFYNSQPG KGYVTDMQHY QEMYQQSINE PEKFFDKMAK	60	
EYLNHWADPYT KVQSGSLNNNG DVAWFLNGKL NASYNCVDRH AFANPDKPAL IYEADDESDN	120	
KIITFGEELLR KVSQIAVGVLK SWGVKKGDTV AIYLPMPIMEA VIAMLAVERI GAIHSVVFAG	180	
FSAGSLKDRV VDANSKVIT CDEGKRGKKT INTKKIVDEG LNGVDSLVSRI LVFQRTGTEG	240	
IPMKAGRDYW WHEEAQKQRTY YLPPVSCDAE DPPLFLLYTSG STGSPKGVHV TTGGYLLGAA	300	
LTRYVFDIH PEDVLFITAGD VGWITCHTYA LYGPLTLCTA SIIIFESTPAY PDYGRYWRII	360	
QRHKATHFYV APTALRLIKE VGEAEIAKYD TSSLRVLGSV GEPISPDLWS WYHEKVGKNK	420	
CVICDMWQT ESGSHLIAPL AGAVPTKPGS ATPVFFGINA CIIDPVTGVE LEGNDVEGVL	480	
AVKSPWPMSA RSVWNHHDRY MDTYLKPKPG HYPTGDGAGR DHDGYYWIRG RVDDVVNVSG	540	
HRLSTSEIEA SISNHENVSE AAVVGIPDEL TGQTVVAYVS LKDGYLQNNA TEGDAEHITP	600	
DNRRELILO VRGEIGPFAS PKITILVRLDL PRTRSGKIMR RVLRKVASNE AEQLGDLTTL	660	
ANPEVVPAAI SAVENQFFSQ KKK	683	
SEQ ID NO: 50	moltype = DNA length = 2052	
FEATURE	Location/Qualifiers	
source	1..2052	
	mol_type = other DNA	
	organism = <i>Saccharomyces cerevisiae</i>	
SEQUENCE: 50		
atgacaatca aggaacataa agtagttat gaagctcaca acgtaaaggc tcttaaggct	60	
cctcaacatt ttcaacacag ccaacccggc aagggttacg ttactgatat gcaacattat	120	
caagaatgtt atcaacaatc tataaatcg ccagaaaaat tctttgataa gatggctaag	180	
gaataacttcg atggatgc tccataacc accatgttcaat ctgggttattt gaaacatgtt	240	
gatgttgcgat gtttttgaa cggtaatttgc aatgcattcat acaattgtgt tgacagacat	300	
gcctttgtca atcccgcata gccagcttgc atctatcgaa ctgatgcga atccgacac	360	
aaaatcatca cattttgtgtt attactcaga aaagtttccc aaatcgcttgg tgctttaaaa	420	
agctggggcg ttaagaaagg tgacacatgt gctatcttatt tgccatgtat tccagaacgc	480	
gtcatttgctca tggtggctgt ggctgttattt ggtgttattt actctgttgtt ctttgtctgg	540	
ttctccgtgt gtccgttgcgaa agatcggttc gttgacgtca attcttaaagt ggtcatca	600	
tgtgtatggat gtaaaatggg tggtaagggc atcaacacta aaaaatgtt tgacqaaagg	660	
ttgaacggatc tcgatgttgc ttccgttgc tttgttttc aagaacttgg tactcgaaagg	720	
attccaatggat aggccggtag agattactgg tggcatgagg aggccgttca gcaagaaact	780	
taccttaccc tcgtttcatg tgacgttgc gatccttat ttttattata cacttccgg	840	
tccactgtt ccaccaaaaggc tgctgttgc actacagggtt gtttattattt aggtgcgcgt	900	
ttaacaacta gatacggtt ttatccatc ccagaagatg ttcttccac tgccgggtgac	960	
gtcggctgtca tcacgggtca cacctatgtt ctatatgttca cattaacctt gggatccgc	1020	
tcaataatatt tcgaatccac tcctgttgc acgtttttttt gtagatattt gagaattatc	1080	
caacatcgatc accgttccatc ttccatgttgc gtcgttgcactt aatcaaactgtt	1140	
gttaggttgcgatccaaatccatcacttccatg acttccatgatc ttatccatgatc gtttccgtt	1200	
gttgcggatccaaatccatcacttccatg ctatccatgatc gtttccgtt gtttccgtt	1260	
tgtgttgcattt gtgacactat gttggcaaaaca gagtctgttgc ttcattttat tgctcttt	1320	
gcagggttgcgatccaaatccatcacttccatg acttccatgatc ttatccatgatc gtttccgtt	1380	
tgtatccatgttgc accctgttgc aggtgtggaa tttagaaggta atgatgttgc aggtgttgc	1440	
gcgggttataat caccatggcc atcaatccatg acttccatgatc ttatccatgatc gtttccgtt	1500	
atggataactt acttgcggatccaaatccatcacttccatg ctatccatgatc gtttccgtt	1560	
gatcatgtatc gtactactgttgc accctgttgc aggtgttgc acttccatgatc ttatccatgatc	1620	
catagatttttccatcacttccatg ctatccatgatc gtttccgtt gtttccgtt	1680	
gctgtgttgc accctgttgc aggtgttgc accctgttgc accctgttgc atatccatgatc	1740	
ctaaaatggatc gttatccatcacttccatg ctatccatgatc gtttccgtt gtttccgtt	1800	
gataatccatgatc gttatccatgacttccatg ctatccatgatc gtttccgtt gtttccgtt	1860	
ccaaaaatccatgatc gttatccatgacttccatg ctatccatgatc gtttccgtt gtttccgtt	1920	
agagttcttgc accctgttgc accctgttgc accctgttgc atatccatgatc gtttccgtt	1980	
gccaacccatgatc gttatccatgacttccatg ctatccatgatc gtttccgtt gtttccgtt	2040	
aaaaagaaat aa	2052	
SEQ ID NO: 51	moltype = AA length = 571	
FEATURE	Location/Qualifiers	
source	1..571	
	mol_type = protein	
	organism = <i>Millerozyma farinose</i>	
SEQUENCE: 51		
MGFELWGRTN TGGLRGRPLR VAITAVATTG FSLFGYDQGL MSGIITGTEF NEEFPPTWSK	60	
PHYNASEKRH ATVVQGAUTA CYEIGCFFGA LFALVRGDRI GRRPLVIVGA VLIIIGTVIS	120	
TAAFGEHWGL QOFVIGRVIT GIGNGMNTAT IPVWQSEISR PENRGKLVNL EGSVIAIGTF	180	
VAYWIDFGLS YVNSSVQWRF PVAFQIVFAA GLLGGILFMP ESPRWLLAHLG KKEQAHIVLG	240	
ALNDLDPNDD HVLAESTVIT DAINRFSRSQ LGFKELMSGG KNQHFRMVI GSSTOFFQQF	300	
TGCNAAIYYS TVLFEETIFV GDRRLSLVMG GVFASVYALA TIPSFFLVDK LGRRNLFLIG	360	
ATGQALSTFTI TFACLINPTK QNAKGAAVGI FLFITFFFATL ILPLPWLYPP EINPLRTRTV	420	
ASAVSTCTNW LTNFAVVMFT PIFINDAQWG CYLFFACLNYY AFIPVIFWFY PETAGRSLEE	480	
IDIIIFAKAYT DGRPPWRVAA TMPHLSLKEQ EEQGMQLGLY DNEAEKQKFE QTENLMSSS	540	
SAKLPEEGSN VNENENENTN EKDQTPKPTD V	571	

-continued

```

SEQ ID NO: 52      moltype = DNA  length = 1716
FEATURE           Location/Qualifiers
misc_feature      1..1716
                    note = Encoding SEQ ID NO: 51 and codon-optimized for
                           expression in Saccharomyces cerevisiae
source            1..1716
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 52
aaggggattcg aactttgggg aaggaccaa acaggtgtt tgagaggtag accttgcgt 60
gttgcataccat ccgttgtc aactactgtt ttctccctt tcggatata tcagggttg 120
atgtctgtta ttattaccgg tactgaattt aacgaggagt tccctcaac ctggccaaag 180
ccacattaca accgtctca gaagagacat gctactgtt ttcaagggtc tgttacagct 240
tgttacgaaa ttgggtt cttcggtgtt cttttgtt tggttagagg tgacaggatc 300
ggtagacgtc cacttgtcat tggtgtgtt gtcttataca tcattgttca tgttatttc 360
actgtgttcc ttggtaaca ctggggttt ggtcaattcg ttattggtag agtttattact 420
gttattgttta accgttatgaa cacagcaact atcccagtct ggcaatctga gatctctcg 480
ccagaaaaaca gaggttaaggt agtcaacttga gaaggttca tgattgttccat tggtacttcc 540
gttgcattact ggattgtt cgggtctcc tacgttaaca gctctgtaca atggaggatc 600
cctgtgttgc tccaaattgt ttttgcgtt ggacttctt gaggatttct tttcatggcc 660
gagtctccat gatgttgcgat cgctcatgg aagaaggagc aagcacatc agtcttaggt 720
gctttgaatg atctcgaccat taatgttgc catgtcttgc ctggagatc tgttattacc 780
gtatgttattttaa accatgttcc cagggtctca ctgggttca aggaatttgcat tgccgggtt 840
aaaaccaac attttgcgtt aatgttattt ggttcttcca ctcaattttt ccaacagttc 900
actgtgttgc tgcgttgcatt ttactatcca acagttttgc tgcgaagagc cattttcg 960
gttgacagaaa gattgttcc ttggatgtt ggttgcgtt cttccgtata cgcccttgcc 1020
actattccat ctttttttctt agtgcataag cttggataga gaaaattttt cttgattttgt 1080
gttactggc aagtttgc tttcaccattt acatttgcgtt gtttgcataa cccaaacaaag 1140
caaaatgttta aagggtgcgc tggtgttccat ttcttgcgtt tcacccatc cgcctttaca 1200
attttgcattt tgcgttgcatt ttacccatca gaaatcaacc catttgcataa aagaactgtt 1260
gcctctgcgc tttctcatatc tttccatgtt ctttgcataatc ctttgcgttgc tatgttact 1320
ccttattttca ttaacqatgc tcaatgggt ttttgcataatc ttttgcgttgc tatgttact 1380
gctttcatcc cagttatctt ctgggttctac ccagaaactg ctggccgtc ctttgcataa 1440
attgtatatac ttttgcgttgc ggcgttacatc gatggaaagc ctccatggag agtttgcgtt 1500
accatgcac accatgttcc ttacccatca gaaagacaa gaggagcaac gtatgttgc cggactttat 1560
gacaatgttgc ctggaaacaa gaaatgttgc gaaatccatca acttgcataatc tttagtgc 1620
tctgcgttgc ttcctgttgc gggatcttca gtaaacatca gatggaaacaa aaacacgaaac 1680
aaaaaggatc aaacacccaa gccaactgtt gtttgc 1716

SEQ ID NO: 53      moltype = AA  length = 569
FEATURE           Location/Qualifiers
source            1..569
                    mol_type = protein
                    organism = Saccharomyces cerevisiae

SEQUENCE: 53
MDLKLNSFK GKFISRTSHW GLTGKKLRYF ITIASMTGFS LFGYDQGLMA SLITGKQFN 60
EFPATKENGD HDRHATVVQG ATTSCYELGC FASLFLVMPM GERIGRKPLI LMGSVITIIG 120
AVISTCAFRRG YWALQOFIIG RRVITGVGTGL NTSTIPVWQS EMSKAENRGL LVNLEGSTIA 180
FGTMIAWID FGTSYTNSSV QWRFPVSMQI VFALFLLAFM IKLPEPRWL ISQSRTEEAR 240
YLVGTLDDAD PNDEEVATTE AMLHDADVNT KHEKHSLSSL FSRGRSQNQLQ RALIAASTQF 300
FQQFTGNCNA IYYSTVLFNK TIKLDYRLLFII IIGGVFATIY ALSTIGSFVIEKLGRRLKF 360
LIGATGQAVS FTITFACLVN ENKENARGAA VGLFLFITFF GLSLLSLPWI YPPEIASMKV 420
RASTNAFSTC TNWLNCNFIAV MFTPIFIGQS GWGCYLFFAV MNLYLIPVIF FFYPETAGRS 480
LEEBIDIIFAK AYEDGTQPWR VANHLPKLSL QEVEDHANAL GSYDDEMEKE DFGEDRVEDT 540
YNQINGDNSS SSSNIKNEDT VNDKANFEG 569

SEQ ID NO: 54      moltype = DNA  length = 1502
FEATURE           Location/Qualifiers
source            1..1502
                    mol_type = other DNA
                    organism = Saccharomyces cerevisiae

SEQUENCE: 54
atcatgacag acacgcaact gtatgtcagg gcgcgttacac cttctgttat gaatttaggt 60
gttgcagg ttctcttattc ttatgttgc ggcgttggaa aatttgcgtt aaaccatcaa 120
ttctgtatggg ttccgtataa accatgttgc gtcgttgcgtt ttcttgcgttgc gatgttgcgtt 180
gttactggc attaggccat ttatgttgc gtcgttgcgtt ctttgcgttgc gtcgttgcgtt 240
tgaatacatac tactattccc ttatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 300
tgcgttgcgtt ttatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 360
attttgggtt ttatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 420
tcgttgcgtt ttatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 480
tgattttctca aagtgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 540
atccaaatgtt tgatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 600
ccaaacacgca gaaatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 660
agagggtttt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 720
ccatatacta ctctactgtt ttatgttgc gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 780

```

-continued

```

tgatcatagg tggggcttc gcaacaatct acgccttac tactatttgt tcatttttc 840
taattaaaaa gtatggtaga cgtaaagctgt ttttattagg tgccacagt caagcagtt 900
cattcacaat tacatgtca tgcttgcgca aaaaaataaa agaaaacgca agaggtgctg 960
ccgtcggtt atttttgtc attacattct ttgtttgtc tttgtatca ttaccatgga 1020
tataccacc agaaaattgca tcaatgaaag ttctgtgcac aacaaacgct ttctccacat 1080
gtactaattg gtgtgttaac ttgtgggtc tcatgttac cccaaatattt attggacagt 1140
ccgggttgggg ttgtactta tttttgtct tatgtatca ttatcattt ccagttatct 1200
tctttttcta ccctgaaacc gcccggaaagaa gtttggagga aatcgacatc atcttgct 1260
aagcatacga ggtatggact caaccatgga gagttgtcaa ccatttgccc aagttatccc 1320
tacaagaatgt cgaatgtcat gccaatgtcat ttggcttc tgacgacgaa atggaaaaag 1380
aggactttgg tgaatgataga gtagaagaca ctatataacca aattaacgca gataattcgt 1440
ctagttcttc aaacatcaaa aatgaagata cagtgaacga taaagcaaat tttgagggtt 1500
ga 1502

SEQ ID NO: 55 moltype = AA length = 128
FEATURE Location/Qualifiers
source 1..128
mol_type = protein
organism = Lactobacillus paracasei
SEQUENCE: 55
MLRKFKTID GKTYLVEEMEE IGGAPAAQPA PAAPAAATPTP APAAPAAPAP AAPVAPTGE 60
EVVTAPMPGT VTKILVKDGD AVTNQPLMI LEAMKMENEI VAPKAGTIGQ VFATLNQN 120
SGDNLISI 128

SEQ ID NO: 56 moltype = DNA length = 390
FEATURE Location/Qualifiers
source 1..390
mol_type = other DNA
organism = Lactobacillus paracasei
SEQUENCE: 56
atgttgagaa aattcaagat cacgattgtat gggaaaaacctt atttgggtcgaa aatggaaagaa 60
atggcggtt cgccagccgc ccagcctgcg cccggccgcac cagccccac gccgacacccg 120
gcaccggccgc caccagctgc gccagcacctt gcagctccgg ttgcggccac tggggaaagg 180
gaagttgtca ctgcaccaat gccaggcactt gtcaccaaga ttttggtaa agacggtgat 240
gcagtcaacgg aaaaatcagcc gctgtatgtt ctgaaagccaa tgaatgtgaa aacqaaattt 300
gtggcgcccta aggccgttac catcgccacgat ttgtttgcac cacttaacca gaatgtcaat 360
tccggcgacatactcatcgat cattatataa 390

```

1. A combination of a first microbial host cell having a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product and a second microbial host cell having a second metabolic pathway comprising one or more second enzymes for converting at least in part the first metabolic product into a second metabolic product, wherein:

- at least one of the first microbial host cell or the second microbial host cell is recombinant;
- at least one of the first microbial host cell or the second microbial host cell is a bacterial host cell;
- at least one of the first microbial host cell or the second microbial host cell is a yeast host cell;
- when the first microbial host cell is a recombinant first microbial host cell, the recombinant first microbial host cell has increased activity in the first metabolic pathway, when compared to a corresponding native first microbial host cell, for producing the first metabolic product; and

when the second microbial host cell is a recombinant second microbial host cell, the recombinant second microbial host cell has increased activity in the second metabolic pathway, when compared to a corresponding native second microbial host cell, for converting at least in part the first metabolic product into the second metabolic product.

2. The combination of claim 1, wherein the first microbial host cell is a bacterial host cell and the second microbial cell is a yeast host cell.

3. The combination of claim 2, wherein at least one of the one or more first enzymes are native enzymes and/or at least one of the one or more second enzymes are heterologous enzymes.

4. The combination of claim 2, wherein the first metabolic product is an organic acid or an ester thereof and/or the second metabolic product is ethanol and wherein:

- the one or more first enzymes comprises a citrate lyase; and/or
- the one or more second enzymes comprise one or more of: one or more heterologous polypeptides having acetaldehyde dehydrogenase activity, and/or one or more heterologous polypeptides having acetyl-coA synthetase activity.

5. The combination of claim 4, wherein (i) the yeast host cell is the recombinant yeast host cell and (ii) the heterologous polypeptide having acetaldehyde dehydrogenase activity is an acetylating dehydrogenase (AADH) or a bifunctional acetaldehyde/alcohol dehydrogenase (ADHE), the one or more second enzymes comprising an heterologous polypeptide having NADP⁺-dependent alcohol dehydrogenase activity and/or an heterologous polypeptide having acetyl-coA synthetase activity.

6. The combination of claim 1, wherein the first microbial host cell is a yeast host cell and the second microbial host cell is a bacterial host cell.

7. The combination of claim 6, wherein at least one of the one or more first enzymes is a heterologous enzyme and/or at least one of the one or more second enzymes is a heterologous enzyme.

8. The combination of claim **6**, wherein the first metabolic product is a carbohydrate and/or the second metabolic product is ethanol.

9. The combination of claim **8**, wherein (i) the carbohydrate is trehalose and (ii) the one or more first enzymes comprises trehalose-6-phosphate synthase and/or trehalose-6-phosphate phosphatase.

10. The combination of claim **8**, wherein (i) the carbohydrate is mannitol, and (ii) the one or more first enzymes comprises mannitol-1-phosphate 5-dehydrogenase, the one or more second enzymes comprise a product of at least one gene from a mannitol utilization operon, and/or the one or more second enzymes comprises a mannitol transporter.

11. The combination of claim **8**, wherein (i) the carbohydrate is sorbitol and (ii) the one or more first enzymes comprises sorbitol-6-phosphate dehydrogenase, and/or the one or more second enzymes comprises a product of at least one gene from a sorbitol utilization operon.

12. The combination of claim **8**, wherein (i) the carbohydrate is glycerol, (ii) the one or more second enzymes comprise at least one of a glycerol dehydrogenase, a dihydroxyacetone kinase, a glycerol kinase, a glycerol-3-phosphate dehydrogenase, and/or a glycerol facilitator.

13. The combination of claim **12**, wherein the yeast host cell has increased activity, when compared to the corresponding native yeast host cell, in an NADP⁺-dependent aldehyde dehydrogenase and/or in a phosphoketolase.

14. The combination of claim **1**, wherein the yeast host cell is from *Saccharomyces* sp. or from *Saccharomyces cerevisiae*.

15. The combination of claim **1**, wherein the bacterial host cell further comprises a third metabolic pathway comprising one or more third enzymes for producing a third metabolic product.

16. The combination of claim **15**, wherein the third metabolic product is ethanol and the one or more third enzymes for producing the third metabolic product comprises a pyruvate decarboxylase and/or an alcohol dehydrogenase; and/or wherein the bacterial host cell has a decreased lactate dehydrogenase activity when compared to the corresponding native bacterial host cell.

17. The combination of claim **1**, wherein the bacterial host cell is a lactic acid bacteria.

18. The combination of claim **17**, wherein the bacterial host cell is from *Lactobacillus* sp. or from *Lactobacillus paracasei*.

19. A process for converting a biomass into a fermentation product, the process comprises contacting the biomass with the combination of claim **1** under condition to allow conversion of at least a part of the biomass into the fermentation product.

20. A commercial package comprising:

(i) a combination of a first microbial host cell having a first metabolic pathway comprising one or more first enzymes for producing a first metabolic product and a second microbial host cell having a second metabolic pathway comprising one or more second enzymes for converting at least in part the first metabolic product into a second metabolic product, wherein:

at least one of the first microbial host cell or the second microbial host cell is recombinant;

at least one of the first microbial host cell or the second microbial host cell is a bacterial host cell;

at least one of the first microbial host cell or the second microbial host cell is a yeast host cell;

when the first microbial host cell is a recombinant first microbial host cell, the recombinant first microbial host cell has increased activity in the first metabolic pathway, when compared to a corresponding native first microbial host cell, for producing the first metabolic product; and

when the second microbial host cell is a recombinant second microbial host cell, the recombinant second microbial host cell has increased activity in the second metabolic pathway, when compared to a corresponding native second microbial host cell, for converting at least in part the first metabolic product into the second metabolic product; and

(ii) instructions to perform a process for converting a biomass into a fermentation product, the process comprises contacting the biomass with the combination of (i) under condition to allow conversion of at least a part of the biomass into the fermentation product.

* * * * *