

FERMENTATIVE PRODUCTION OF 2-PHENYLETHANOL FROM GASEOUS SUBSTRATES

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 [0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/991,428 filed March 18, 2020, the entirety of which is incorporated herein by reference.

GOVERNMENT RIGHTS

[0002] This invention was made with government support under Cooperative Agreement DE-EE0007728 awarded by the Department of Energy.

10 FIELD OF THE DISCLOSURE

[0003] This application relates to methods for production of 2-phenylethanol by microbial fermentation of substrates comprising carbon dioxide (CO₂), carbon monoxide (CO), and/or hydrogen (H₂) and genetically modified microorganisms for use in such methods.

BACKGROUND

- 15 [0004] Mitigation of impending climate change requires drastic reductions in emissions of greenhouse gases (GHGs), such as those generated through the burning of fossil fuels like coal and oil. Although sustainable sources of chemicals and transportation fuels are currently insufficient to significantly displace our dependence on fossil carbon, gas fermentation has recently emerged as an alternative platform for the biological fixation of such gases such as
- 20 CO₂, CO, and/or H₂ into sustainable fuels and chemicals. In particular, gas fermentation technology can utilize a wide range of feedstocks including gasified organic matter (e.g., municipal solid waste or agricultural waste) or industrial waste gases (e.g., from steel mills or oil refineries) to produce ethanol, jet fuel, and a variety of other products. Gas fermentation alone could displace 30% of crude oil use and reduce global CO₂ emissions by 10%, but, as
- 25 with any disruptive technology, many technical challenges must be overcome before this potential is fully achieved.

- [0005] 2-phenylethanol (2-PE) is a commodity chemical with a market of 6010 mt/yr in food, fragrance and flavor industries for its “rose-like” aroma and 900 mt/yr in chemical industries as an ester intermediate and, theoretically, in styrene production. Currently, 2-PE is procured
- 30 from natural sources for its application in the food, fragrance, and flavor industries, or it is

synthesized by chemical means for other applications. There is thus a need for improved methods of 2-PE production that alleviate dependence on natural and petrochemical processes.

5 SUMMARY

[0006] In one aspect, the disclosure provides a microorganism capable of producing 2-phenylethanol, wherein the microorganism comprises a heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde and a heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol.

10 [0007] In some embodiments, the heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde is decarboxylase and the heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol is phenylacetaldehyde reductase. In particular, the decarboxylase may be a phenylpyruvate-specific decarboxylase.

[0008] In some embodiments, the microorganism is a C1-fixing microorganism, a Wood-
15 Ljungdahl microorganism, and/or a bacterium. In some embodiments, the microorganism is a member of the genus *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, or *Thermoanaerobacter*.

[0009] In some embodiments, the microorganism is natively capable of producing phenylpyruvate. In some embodiments, the microorganism comprises a Wood-Ljungdahl
20 pathway that converts CO, CO₂, and/or H₂ to acetyl-CoA.

[0010] In some embodiments, the microorganism comprises one or more of: a native enzyme that converts acetyl-CoA to pyruvate; a native enzyme that converts pyruvate to phosphoenolpyruvate; a native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate; a native enzyme that
25 converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquinate; a native enzyme that converts 3-dehydroquinone to 3-dehydroshikimate; a native enzyme that converts 3-dehydroshikimate to shikimate; a native enzyme that converts shikimate to shikimate 3-phosphate; a native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate; a native enzyme that converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate; or a native enzyme that converts chorismate to phenylpyruvate.
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[0011] In some embodiments, the native enzyme that converts acetyl-CoA to pyruvate is pyruvate:ferredoxin oxidoreductase (PFOR; E.C. 1.2.7.1); the native enzyme that converts pyruvate to phosphoenolpyruvate is pyruvate phosphate dikinase (PPDK; E.C.2.7.9.1); the native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase (DAHPS or DAHP synthase; E.C.2.5.1.54); the native enzyme that converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquininate is 3-dehydroquininate synthase (DHQS; E.C. 4.2.3.4); the native enzyme that converts 3-dehydroquininate to 3-dehydroshikimate is 3-dehydroquininate dehydratase (DHQ; E.C. 4.2.1.10); the native enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase (SDH; E.C. 1.1.1.25); the native enzyme that converts shikimate to shikimate 3-phosphate is shikimate kinase (SK; E.C. 2.7.1.71); the native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate is 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS or EPSP synthase; E.C. 2.5.1.19); the native enzyme that converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate is chorismate synthase (CS; E.C. 4.2.3.5); or the native enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase (E.C. 5.4.99.5)/prephenate dehydratase (E.C.4.2.1.51).

[0012] In some embodiments, the microorganism further comprises one or more of: a heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate; a heterologous enzyme that converts 3-dehydroshikimate to shikimate; or a heterologous enzyme that converts chorismate to phenylpyruvate.

[0013] In some embodiments, the heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase; the heterologous enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase; or the heterologous enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

[0014] In some embodiments, the microorganism comprises:

- (1) ecAroE, kivD, lePAR, pheA2, and aroG;
- (2) ecAroE, abPPDC, ecPAR, pheA1, and aroG;

- (3) cgAroE, abPPDC, lePAR, pheA2, and aroG;
- (4) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- (5) cgAroE, kivD, rrPAR, pheA2, and aroG;
- (6) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- 5 (7) pheA1, aroG, abPPDC, and ecPAR;
- (8) egAroE, abPPDC, lePAR, pheA1, and aroG;
- (9) ecAroE, abPPDC, ecPAR, pheA2, and aroG;
- (10) cgAroE, aro10, ecPAR, pheA2, and aroG;
- (11) cgAroE, abPPDC, lePAR, pheA1, and aroG;
- 10 (12) cgAroE, aro10, lePAR, pheA2, and aroG;
- (13) ecAroE, abPPDC, lePAR, pheA1, and aroG;
- (14) ecAroE, aro10, ecPAR, pheA2, and aroG;
- (15) ecAroE, aro10, lePAR, pheA2, and aroG;
- (16) cgAroE, abPPDC, lePAR, pheA1, and aroG;
- 15 (17) cgAroE, aro10, rsPAR, pheA2, and aroG;
- (18) cgAroE, abPPDC, blPAR, pheA2, and aroG;
- (19) ecAroE, kivD, blPAR, pheA2, and aroG;
- (20) cgAroE, aro10, blPAR, pheA2, and aroG;
- (21) ecAroE, abPPDC, blPAR, pheA2, and aroG;
- 20 (22) ecAroE, abPPDC, adh6, pheA1, and aroG;
- (23) cgAroE, abPPDC, adh6, pheA2, and aroG;
- (24) cgAroE, abPPDC, ecPAR, pheA2, and aroG;
- (25) ecAroE, aro10, rsPAR, pheA2, and aroG;
- (26) ecAroE, aro10, adh6, pheA2, and aroG; or
- 25 (27) ecAroE, abPPDC, ecPAR, pheA2, and aroG.

[0015] In some embodiments, the microorganism comprises the heterologous genes and promoters of combinatorial strain 2C03, 1C10, 2C54, 2C09, 2C47, 2C55, 2C53, H57, 1C47, 2C10, 2A18, 1C54, 2C01, 1C31, 2B17, 2C52, 1C09, H18, H58, 2C27, 2D22, 2D07, 2D24, 2D03, 1C29, 2B26, 2C38, 2A27, 2C12, or 2B27.

- 30 **[0016]** In some embodiments, the microorganism ferments a gaseous substrate comprising CO, CO₂, and/or H₂ to produce 2-phenylethanol. In some embodiments, the gaseous substrate comprises syngas or industrial waste gas.

[0017] In some embodiments, the microorganism does not produce any other C3+ alcohols. In some embodiments, the microorganism does not produce any other C3, C4, C5, C6, C7, C8, C9, or C10 alcohols.

5 [0018] In another aspect, the disclosure provides a method of producing 2-phenylethanol comprising culturing the microorganism in the presence of a gaseous substrate. In some embodiments, the gaseous substrate comprises a C1-carbon source comprising CO, CO₂, and/or H₂. In some embodiments, the gaseous substrate comprises syngas or industrial waste gas.

10 [0019] Specific embodiments of the disclosure will become evident from the following more detailed description of certain embodiments and the claims.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0020] **Figure 1** shows the metabolic pathway leading to the biosynthesis of 2-phenylethanol by *Clostridium autoethanogenum* from gas fermentation. Enzymatic steps that require
15 heterologous expression are the decarboxylase and phenylacetaldehyde reductase (PAR) steps. The dashed arrow depicts the multi-enzymatic core metabolic pathway in *C. autoethanogenum*. Carbon and hydrogen are assimilated to acetyl-CoA by the Wood-Ljungdahl pathway which is then converted to pyruvate by a pyruvate:ferredoxin oxidoreductase. Aromatic amino acid biosynthesis starts with phosphoenolpyruvate and
20 erythrose-4-phosphate, which are biosynthesized from pyruvate by essential metabolic pathways like gluconeogenesis and pentose phosphate pathway. 2-phenylpyruvate can be derived from the shikimate pathway and by aminotransferase reactions between phenylalanine and keto acids. Enzymes that were heterologously expressed or overexpressed in the Examples disclosed herein are marked with a tick mark. NAD(P)H comprises NADPH
25 and NADH.

[0021] **Figures 2A-2B** show autotrophic growth of *Clostridium autoethanogenum* in the presence of different amounts of 2-PE in Schott bottles. **Figure 2A**: Growth profile; **Figure 2B**: 2-PE concentration measured by GC-FID. Unc = Unchallenged; N = 2; Error bar = S.D.

30 [0022] **Figure 3A** shows the growth profile and **Figure 3B** the end-point alcohol production titer during autotrophic growth using 150 kPa of synthetic gas blend (50% CO, 10% H₂, 30% CO₂ and 10% N₂) in Schott bottles between the three decarboxylase strains: *abPPDC*, *aro10* and control *kivD_la*. N = 3; Error bar = S.D.

[0023] **Figures 4A-4C** show overexpression of phenylacetaldehyde reductase (PAR) in *Clostridium autoethanogenum* for 2-PE production. **Figure 4A**: The species origin, amino acid length, cofactor specificity, and reference of employed PAR. **Figure 4B**: Phylogenetic tree of PAR variants using amino acid sequence. The scale bar corresponds to 0.2 change per amino acid. **Figure 4C**: Normalized 2-PE production by strains with different PAR variants in 12-well plates after 7 days of incubation in the presence of 200 kPa synthetic gas blend (50% CO, 10% H₂, 30% CO₂ and 10% N₂); N=3-5; Error bar = S.D.

[0024] **Figure 5** shows combinatorial assembly of 2-PE pathway genes using the Golden Gate method.

10 [0025] **Figure 6** shows gene and promoter variants that were employed for the two 2-PE combinatorial libraries described in Example 4.

[0026] **Figures 7A-C** show characterization of 162 2-PE combinatorial strains in 12-well plates with 200 kPa synthetic gas blend (50% CO, 10% H₂, 30% CO₂, and 10% N₂). **Figure 7A**: 2-PE titer relative to control strain sFA212; **Figure 7B**: 2-PE titer normalized by biomass concentration; **Figure 7C**: Biomass concentration; Incubation duration = 8-10 days; N = 2; Error bar = S.D.

15 [0027] **Figures 8A-E** show comparisons of end-point 2-PE titers in combinatorial strains according to: number of plasmids (**Figure 8A**); bi-functional chorismate mutase/prephenate dehydratase variants (**Figure 8B**); decarboxylase variants (**Figure 8C**); shikimate dehydrogenase variants (**Figure 8D**); and phenylacetaldehyde reductase variants (**Figure 8E**). Incubation duration = 8-10 days; Error bar = S.D.

20 [0028] **Figures 9A-B** show characterization of 12 combinatorial strains in continuous CSTR fermentation using a synthetic gas blend (40% CO, 20% H₂, 20% CO₂, and 20% N₂). 2.9-fold increase in 2-PE titer relative to reference strain sFA212 was observed in strain 1C29. 10 strains achieved higher 2-PE yield when compared to the reference strain.

[0029] **Figures 10A-B** show 2-PE production of combinatorial strain 1C29 in two continuous CSTR runs with nitrogen limitation.

25 [0030] **Figures 11A-C** show results of testing the effect of pre-treated corn stover-derived syngas (23.3% H₂, 38.7% CO, 19.6% CO₂, and 18.4% N₂) in continuous CSTR fermentation using strain sFA212. **Figure 11A**: metabolite profile analyzed by HPLC; **Figure 11B**: gas profile analyzed by GC-TCD (negative = uptake); **Figure 11C**: 2-PE profile by analyzed GC-FID. Real syngas was used between day 6.9 and 10.9 (total of 4 days).

DETAILED DESCRIPTION

[0031] Disclosed herein are methods and compositions for *de novo* biosynthesis of 2-phenylethanol (2-PE) from syngas, which have potential to alleviate dependence on natural and petrochemical processes to manufacture 2-PE.

Definitions

[0032] Unless otherwise defined, the following terms as used throughout this specification are defined as follows:

[0033] The term “fermentation” should be interpreted as a metabolic process that produces chemical changes in a substrate. For example, a fermentation process receives one or more substrates and produces one or more products through utilization of one or more microorganisms. The term “fermentation,” “gas fermentation” and the like should be interpreted as the process which receives one or more substrate, such as syngas produced by gasification and produces one or more product through the utilization of one or more C1-fixing microorganism. Preferably the fermentation process includes the use of one or more bioreactor. The fermentation process may be described as either “batch” or “continuous”. “Batch fermentation” is used to describe a fermentation process where the bioreactor is filled with raw material, e.g. the carbon source, along with microorganisms, where the products remain in the bioreactor until fermentation is completed. In a “batch” process, after fermentation is completed, the products are extracted, and the bioreactor is cleaned before the next “batch” is started. “Continuous fermentation” is used to describe a fermentation process where the fermentation process is extended for longer periods of time, and product and/or metabolite is extracted during fermentation. Preferably the fermentation process is continuous.

[0034] The term “non-naturally occurring” when used in reference to a microorganism is intended to mean that the microorganism has at least one genetic modification not found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Non-naturally occurring microorganisms are typically developed in a laboratory or research facility.

[0035] The terms “genetic modification,” “genetic alteration,” or “genetic engineering” broadly refer to manipulation of the genome or nucleic acids of a microorganism by the hand of man. Likewise, the terms “genetically modified,” “genetically altered,” or “genetically

engineered” refers to a microorganism containing such a genetic modification, genetic alteration, or genetic engineering. These terms may be used to differentiate a lab-generated microorganism from a naturally occurring microorganism. Methods of genetic modification of include, for example, heterologous gene expression, gene or promoter insertion or deletion, nucleic acid mutation, altered gene expression or inactivation, enzyme engineering, directed evolution, knowledge-based design, random mutagenesis methods, gene shuffling, and codon optimization.

[0036] Metabolic engineering of microorganisms, such as *Clostridia*, can tremendously expand their ability to produce many important fuel and chemical molecules other than native metabolites, such as ethanol. In recent years several different methods for genome engineering for *Clostridia* have been developed including intron-based methods (ClosTron) (Kuehne, *Strain Eng: Methods and Protocols*, 389-407, 2011), allelic exchange methods (ACE) (Heap, *Nucl Acids Res*, 40: e59, 2012; Ng, *PLoS One*, 8: e56051, 2013), Triple Cross (Liew, *Frontiers Microbiol*, 7: 694, 2016), methods mediated through I-SceI (Zhang, *Journal Microbiol Methods*, 108: 49-60, 2015), MazF (Al-Hinai, *Appl Environ Microbiol*, 78: 8112-8121, 2012), or others (Argyros, *Appl Environ Microbiol*, 77: 8288-8294, 2011), Cre-Lox (Ueki, *mBio*, 5: e01636-01614, 2014), and CRISPR/Cas9 (Nagaraju, *Biotechnol Biofuels*, 9: 219, 2016). However, it remains extremely challenging to iteratively introduce more than a few genetic changes, due to slow and laborious cycling times and limitations on the transferability of these genetic techniques across species. Furthermore, we do not yet sufficiently understand C1 metabolism in *Clostridia* to reliably predict modifications that will maximize C1 uptake, conversion, and carbon/energy/redox flows towards product synthesis. Accordingly, introduction of target pathways in *Clostridia* remains a tedious and time-consuming process.

[0037] “Recombinant” indicates that a nucleic acid, protein, or microorganism is the product of genetic modification, engineering, or recombination. Generally, the term “recombinant” refers to a nucleic acid, protein, or microorganism that contains or is encoded by genetic material derived from multiple sources, such as two or more different strains or species of microorganisms.

[0038] “Wild type” refers to the typical form of an organism, strain, gene, or characteristic as it occurs in nature, as distinguished from mutant or variant forms.

[0039] “Endogenous” or “native” refers to a nucleic acid or protein that is present or expressed in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, an endogenous gene is a gene that is natively present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. In one embodiment, the expression of an endogenous gene may be controlled by an exogenous regulatory element, such as an exogenous promoter.

[0040] “Exogenous” refers to a nucleic acid or protein that originates outside the microorganism of the disclosure. For example, an exogenous gene or enzyme may be artificially or recombinantly created and introduced to or expressed in the microorganism of the disclosure. An exogenous gene or enzyme may also be isolated from a heterologous microorganism and introduced to or expressed in the microorganism of the disclosure. Exogenous nucleic acids may be adapted to integrate into the genome of the microorganism of the disclosure or to remain in an extra-chromosomal state in the microorganism of the disclosure, for example, in a plasmid.

[0041] “Heterologous” refers to a nucleic acid or protein that is not present in the wild-type or parental microorganism from which the microorganism of the disclosure is derived. For example, a heterologous gene or enzyme may be derived from a different strain or species and introduced to or expressed in the microorganism of the disclosure. The heterologous gene or enzyme may be introduced to or expressed in the microorganism of the disclosure in the form in which it occurs in the different strain or species. Alternatively, the heterologous gene or enzyme may be modified in some way, e.g., by codon-optimizing it for expression in the microorganism of the disclosure or by engineering it to alter function, such as to reverse the direction of enzyme activity or to alter substrate specificity.

[0042] The terms “polynucleotide,” “nucleotide,” “nucleotide sequence,” “nucleic acid,” and “oligonucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof.

Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A

polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides or nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after
5 polymerization, such as by conjugation with a labeling component.

[0043] As used herein, “expression” refers to the process by which a polynucleotide is transcribed from a DNA template (such as into and mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred
10 to as “gene products.”

[0044] The terms “polypeptide”, “peptide,” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide
15 bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein, the term “amino acid” includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

[0045] “Enzyme activity,” or simply “activity,” refers broadly to enzymatic activity,
20 including, but not limited, to the activity of an enzyme, the amount of an enzyme, or the availability of an enzyme to catalyze a reaction. Accordingly, “increasing” enzyme activity includes increasing the activity of an enzyme, increasing the amount of an enzyme, or increasing the availability of an enzyme to catalyze a reaction. Similarly, “decreasing” enzyme activity includes decreasing the activity of an enzyme, decreasing the amount of an
25 enzyme, or decreasing the availability of an enzyme to catalyze a reaction.

[0046] “Codon optimization” refers to the mutation of a nucleic acid, such as a gene, for optimized or improved translation of the nucleic acid in a particular strain or species. Codon optimization may result in faster translation rates or higher translation accuracy.

[0047] “Overexpressed” refers to an increase in expression of a nucleic acid or protein in the
30 microorganism of the disclosure compared to the wild-type or parental microorganism from which the microorganism of the disclosure is derived. Overexpression may be achieved by

any means known in the art, including modifying gene copy number, gene transcription rate, gene translation rate, or enzyme degradation rate.

[0048] The term “variants” includes nucleic acids and proteins whose sequence varies from the sequence of a reference nucleic acid and protein, such as a sequence of a reference
5 nucleic acid and protein disclosed in the prior art or exemplified herein. The methods and compositions of the disclosure may be practiced using variant nucleic acids or proteins that perform substantially the same function as the reference nucleic acid or protein. For example, a variant protein may perform substantially the same function or catalyze substantially the same reaction as a reference protein. A variant gene may encode the same or substantially the
10 same protein as a reference gene. A variant promoter may have substantially the same ability to promote the expression of one or more genes as a reference promoter.

[0049] Such nucleic acids or proteins may be referred to herein as “functionally equivalent variants” or “functional homologues.” By way of example, functionally equivalent variants of a nucleic acid may include allelic variants, fragments of a gene, mutated genes,
15 polymorphisms, and the like. Homologous genes from other microorganisms are also examples of functionally equivalent variants. These include homologous genes in species such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, or *Clostridium ljungdahlii*, the details of which are publicly available on websites such as Genbank or NCBI. Functionally equivalent variants also include nucleic acids whose sequence varies as a result of codon
20 optimization for a particular microorganism. A functionally equivalent variant of a nucleic acid will preferably have at least approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater nucleic acid sequence identity (percent homology) with the referenced nucleic acid. A functionally equivalent variant (*i.e.*, a functional homologue) of a protein will preferably have at least
25 approximately 70%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, approximately 98%, or greater amino acid identity (percent homology) with the referenced protein. The functional equivalence of a variant nucleic acid or protein may be evaluated using any method known in the art.

[0050] Nucleic acid or amino acid sequence identity requires identical amino acid sequences
30 between two aligned sequences. Thus, a candidate sequence sharing 80% nucleic acid or amino acid identity with a reference sequence requires that, following alignment, 80% of the nucleic acids or amino acids in the candidate sequence are identical to the corresponding nucleic acids or amino acids in the reference sequence. Identity according to the present

invention is determined by aid of computer analysis, such as, without limitations, the ClustalW computer alignment program (Higgins et al., Nucleic Acids Res. 22:4673-4680), and the default parameters suggested therein. Using, for example, the ClustalW software with its default settings, the mature (bioactive) part of a query and a reference polypeptide are aligned. The number of fully conserved residues are counted and divided by the length of the reference polypeptide.

[0051] Nucleic acids may be delivered to a microorganism of the disclosure using any method known in the art. For example, nucleic acids may be delivered as naked nucleic acids or may be formulated with one or more agents, such as liposomes. The nucleic acids may be DNA, RNA, cDNA, or combinations thereof, as is appropriate. Restriction inhibitors may be used in certain embodiments. Additional vectors may include plasmids, viruses, bacteriophages, cosmids, and artificial chromosomes. In a preferred embodiment, nucleic acids are delivered to the microorganism of the disclosure using a plasmid. By way of example, transformation (including transduction or transfection) may be achieved by electroporation, ultrasonication, polyethylene glycol-mediated transformation, chemical or natural competence, protoplast transformation, prophage induction, or conjugation. In certain embodiments having active restriction enzyme systems, it may be necessary to methylate a nucleic acid before introduction of the nucleic acid into a microorganism.

[0052] Furthermore, nucleic acids may be designed to comprise a regulatory element, such as a promoter, to increase or otherwise control expression of a particular nucleic acid. The promoter may be a constitutive promoter or an inducible promoter. Ideally, the promoter is a Wood-Ljungdahl pathway promoter, a ferredoxin promoter, a pyruvate:ferredoxin oxidoreductase promoter, an Rnf complex operon promoter, an ATP synthase operon promoter, or a phosphotransacetylase/acetate kinase operon promoter.

[0053] A “microorganism” is a microscopic organism, especially a bacterium, archaeon, virus, or fungus. The microorganism of the disclosure is typically a bacterium. As used herein, recitation of “microorganism” should be taken to encompass “bacterium.”

[0054] A “parental microorganism” is a microorganism used to generate a microorganism of the disclosure. The parental microorganism may be a naturally occurring microorganism (i.e., a wild-type microorganism) or a microorganism that has been previously modified (i.e., a mutant or recombinant microorganism). The microorganism of the disclosure may be modified to express or overexpress one or more enzymes that were not expressed or

overexpressed in the parental microorganism. Similarly, the microorganism of the disclosure may be modified to contain one or more genes that were not contained by the parental microorganism. The microorganism of the disclosure may also be modified to not express or to express lower amounts of one or more enzymes that were expressed in the parental microorganism. In one embodiment, the parental microorganism is *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In a preferred embodiment, the parental microorganism is *Clostridium autoethanogenum* LZ1561, which was deposited with Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) located at Inhoffenstraße 7B, D-38124 Braunschweig, Germany on June 7, 2010 under the terms of the Budapest Treaty and accorded accession number DSM23693. This strain is described in International Patent Application No. PCT/NZ2011/000144, which published as WO 2012/015317.

[0055] The term “derived from” indicates that a nucleic acid, protein, or microorganism is modified or adapted from a different (e.g., a parental or wild-type) nucleic acid, protein, or microorganism, so as to produce a new nucleic acid, protein, or microorganism. Such modifications or adaptations typically include insertion, deletion, mutation, or substitution of nucleic acids or genes. Generally, the microorganism of the disclosure is derived from a parental microorganism. In one embodiment, the microorganism of the disclosure is derived from *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei*. In a preferred embodiment, the microorganism of the disclosure is derived from *Clostridium autoethanogenum* LZ1561, which is deposited under DSMZ accession number DSM23693.

[0056] The microorganism of the disclosure may be further classified based on functional characteristics. For example, the microorganism of the disclosure may be or may be derived from a C1-fixing microorganism, an anaerobe, an acetogen, an ethanologen, a carboxydrotroph, and/or a methanotroph. Table 1 provides a representative list of microorganisms and identifies their functional characteristics.

Table 1

	Wood-Ljungdahl	C1-fixing	Anaerobe	Acetogen	Ethanologen	Autotroph	Carboxydotroph
<i>Acetobacterium woodii</i>	+	+	+	+	+/- ¹	+	-
<i>Alkalibaculum bacchii</i>	+	+	+	+	+	+	+
<i>Blautia producta</i>	+	+	+	+	-	+	+
<i>Butyribacterium methylotrophicum</i>	+	+	+	+	+	+	+
<i>Clostridium aceticum</i>	+	+	+	+	-	+	+
<i>Clostridium autoethanogenum</i>	+	+	+	+	+	+	+
<i>Clostridium carboxidivorans</i>	+	+	+	+	+	+	+
<i>Clostridium coskatii</i>	+	+	+	+	+	+	+
<i>Clostridium drakei</i>	+	+	+	+	-	+	+
<i>Clostridium formicoaceticum</i>	+	+	+	+	-	+	+
<i>Clostridium ljungdahlii</i>	+	+	+	+	+	+	+
<i>Clostridium magnum</i>	+	+	+	+	-	+	+/- ²
<i>Clostridium ragsdalei</i>	+	+	+	+	+	+	+
<i>Clostridium scatologenes</i>	+	+	+	+	-	+	+
<i>Eubacterium limosum</i>	+	+	+	+	-	+	+
<i>Moorella thermautotrophica</i>	+	+	+	+	+	+	+
<i>Moorella thermoacetica</i> (formerly <i>Clostridium thermoaceticum</i>)	+	+	+	+	- ³	+	+
<i>Oxobacter pfennigii</i>	+	+	+	+	-	+	+
<i>Sporomusa ovata</i>	+	+	+	+	-	+	+/- ⁴
<i>Sporomusa silvacetica</i>	+	+	+	+	-	+	+/- ⁵
<i>Sporomusa sphaeroides</i>	+	+	+	+	-	+	+/- ⁶
<i>Thermoanaerobacter kivui</i>	+	+	+	+	-	+	-

¹ *Acetobacterium woodii* can produce ethanol from fructose, but not from gas.

² It has not been investigated whether *Clostridium magnum* can grow on CO.

³ One strain of *Moorella thermoacetica*, *Moorella* sp. HUC22-1, has been reported to produce ethanol from gas.

⁴ It has not been investigated whether *Sporomusa ovata* can grow on CO.

⁵ It has not been investigated whether *Sporomusa silvacetica* can grow on CO.

⁶ It has not been investigated whether *Sporomusa sphaeroides* can grow on CO.

- [0057] “Wood-Ljungdahl” refers to the Wood-Ljungdahl pathway of carbon fixation as described, e.g., by Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008. “Wood-Ljungdahl microorganisms” refers, predictably, to microorganisms containing the Wood-Ljungdahl pathway. Generally, the microorganism of the disclosure contains a native Wood-Ljungdahl pathway. Herein, a Wood-Ljungdahl pathway may be a native, unmodified Wood-Ljungdahl pathway or it may be a Wood-Ljungdahl pathway with some degree of genetic

modification (e.g., overexpression, heterologous expression, knockout, etc.) so long as it still functions to convert CO, CO₂, and/or H₂ to acetyl-CoA.

[0058] “C1” refers to a one-carbon molecule, for example, CO, CO₂, CH₄, or CH₃OH. “C1-oxygenate” refers to a one-carbon molecule that also comprises at least one oxygen atom, for example, CO, CO₂, or CH₃OH. “C1-carbon source” refers a one carbon-molecule that serves as a partial or sole carbon source for the microorganism of the disclosure. For example, a C1-carbon source may comprise one or more of CO, CO₂, CH₄, CH₃OH, or CH₂O₂. Preferably, the C1-carbon source comprises one or both of CO and CO₂. A “C1-fixing microorganism” is a microorganism that has the ability to produce one or more products from a C1 carbon source. Typically, the microorganism of the disclosure is a C1-fixing bacterium. In a preferred embodiment, the microorganism of the disclosure is derived from a C1-fixing microorganism identified in Table 1.

[0059] An “anaerobe” is a microorganism that does not require oxygen for growth. An anaerobe may react negatively or even die if oxygen is present above a certain threshold. However, some anaerobes are capable of tolerating low levels of oxygen (e.g., 0.000001-5% oxygen). Typically, the microorganism of the disclosure is an anaerobe. In some embodiments, the microorganism of the disclosure is derived from an anaerobe identified in Table 1.

[0060] “Acetogens” are obligately anaerobic bacteria that use the Wood-Ljungdahl pathway as their main mechanism for energy conservation and for synthesis of acetyl-CoA and acetyl-CoA-derived products, such as acetate (Ragsdale, *Biochim Biophys Acta*, 1784: 1873-1898, 2008). In particular, acetogens use the Wood-Ljungdahl pathway as a (1) mechanism for the reductive synthesis of acetyl-CoA from CO₂, (2) terminal electron-accepting, energy conserving process, (3) mechanism for the fixation (assimilation) of CO₂ in the synthesis of cell carbon (Drake, *Acetogenic Prokaryotes*, In: *The Prokaryotes*, 3rd edition, p. 354, New York, NY, 2006). All naturally occurring acetogens are C1-fixing, anaerobic, autotrophic, and non-methanotrophic. Typically, the microorganism of the disclosure is an acetogen. In a preferred embodiment, the microorganism of the disclosure is derived from an acetogen identified in Table 1.

[0061] An “ethanologen” is a microorganism that produces or is capable of producing ethanol. Typically, the microorganism of the disclosure is an ethanologen. In a preferred

embodiment, the microorganism of the disclosure is derived from an ethanologen identified in Table 1.

[0062] An “autotroph” is a microorganism capable of growing in the absence of organic carbon. Instead, autotrophs use inorganic carbon sources, such as CO and/or CO₂. Typically, the microorganism of the disclosure is an autotroph. In a preferred embodiment, the microorganism of the disclosure is derived from an autotroph identified in Table 1.

[0063] A “carboxydutroph” is a microorganism capable of utilizing CO as a sole source of carbon and energy. Typically, the microorganism of the disclosure is a carboxydutroph. In a preferred embodiment, the microorganism of the disclosure is derived from a carboxydutroph identified in Table 1.

[0064] A “methanotroph” is a microorganism capable of utilizing methane as a sole source of carbon and energy. In certain embodiments, the microorganism of the disclosure is a methanotroph or is derived from a methanotroph. In other embodiments, the microorganism of the disclosure is not a methanotroph or is not derived from a methanotroph.

[0065] More broadly, the microorganism of the disclosure may be derived from any genus or species identified in Table 1. For example, the microorganism may be a member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyribacterium*, *Clostridium*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*. In particular, the microorganism may be derived from a parental bacterium selected from the group consisting of *Acetobacterium woodii*, *Alkalibaculum bacchii*, *Blautia producta*, *Butyribacterium methylotrophicum*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium coskatii*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Clostridium ragsdalei*, *Clostridium scatologenes*, *Eubacterium limosum*, *Moorella thermautotrophica*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, and *Thermoanaerobacter kivui*.

[0066] These types of microorganisms are known to live in the thermodynamic limit of life (Schuchmann, *Nat Rev Microbiol*, 12: 809-821, 2014) so, prior to the present invention, it was unknown whether they would be capable of synthesizing a C8 aromatic alcohol at all, much less in a meaningful quantity, especially given the potential for 2-PE to have toxic effects on these microorganisms.

[0067] In a preferred embodiment, the microorganism of the disclosure is derived from the cluster of Clostridia comprising the species *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. These species were first reported and characterized by Abrini, *Arch Microbiol*, 161: 345-351, 1994 (*Clostridium autoethanogenum*), Tanner, *Int J System Bacteriol*, 43: 232-236, 1993 (*Clostridium ljungdahlii*), and Huhnke, WO 2008/028055 (*Clostridium ragsdalei*).

[0068] These three species have many similarities. In particular, these species are all C1 fixing, anaerobic, acetogenic, ethanologenic, and carboxydotrophic members of the genus *Clostridium*. These species have similar genotypes and phenotypes and modes of energy conservation and fermentative metabolism. Moreover, these species are clustered in clostridial rRNA homology group I with 16S rRNA DNA that is more than 99% identical, have a DNA G + C content of about 22-30 mol%, are Gram-positive, have similar morphology and size (logarithmic growing cells between 0.5-0.7 x 3-5 μm), are mesophilic (grow optimally at 30-37 °C), have similar pH ranges of about 4-7.5 (with an optimal pH of about 5.5-6), lack cytochromes, and conserve energy via an Rnf complex. Also, reduction of carboxylic acids into their corresponding alcohols has been shown in these species (Perez, *Biotechnol Bioeng*, 110:1066-1077, 2012). Importantly, these species also all show strong autotrophic growth on CO-containing gases, produce ethanol and acetate (or acetic acid) as main fermentation products, and produce small amounts of 2,3-butanediol and lactic acid under certain conditions.

[0069] However, these three species also have a number of differences. These species were isolated from different sources: *Clostridium autoethanogenum* from rabbit gut, *Clostridium ljungdahlii* from chicken yard waste, and *Clostridium ragsdalei* from freshwater sediment. These species differ in utilization of various sugars (e.g., rhamnose, arabinose), acids (e.g., gluconate, citrate), amino acids (e.g., arginine, histidine), and other substrates (e.g., betaine, butanol). Moreover, these species differ in auxotrophy to certain vitamins (e.g., thiamine, biotin). These species have differences in nucleic and amino acid sequences of Wood-Ljungdahl pathway genes and proteins, although the general organization and number of these genes and proteins has been found to be the same in all species (Köpke, *Curr Opin Biotechnol*, 22: 320-325, 2011).

[0070] Thus, in summary, many of the characteristics of *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, or *Clostridium ragsdalei* are not specific to that species, but are rather general characteristics for this cluster of C1 fixing, anaerobic, acetogenic,

ethanologenic, and carboxydotrophic members of the genus *Clostridium*. However, since these species are, in fact, distinct, the genetic modification or manipulation of one of these species may not have an identical effect in another of these species. For instance, differences in growth, performance, or product production may be observed.

5 [0071] “Substrate” refers to a carbon and/or energy source for the microorganism of the disclosure. Typically, the substrate is gaseous and comprises a C1-carbon source, for example, CO, CO₂, and/or CH₄. Preferably, the substrate comprises a C1-carbon source of CO or CO + CO₂. The substrate may further comprise other non-carbon components, such as H₂, N₂, or electrons.

10 [0072] The substrate generally comprises at least some amount of CO, such as about 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mol% CO. The substrate may comprise a range of CO, such as about 20-80, 30-70, or 40-60 mol% CO. Preferably, the substrate comprises about 40-70 mol% CO (e.g., steel mill or blast furnace gas), about 20-30 mol% CO (e.g., basic oxygen furnace gas), or about 15-45 mol% CO (e.g., syngas). In some embodiments, 15 the substrate may comprise a relatively low amount of CO, such as about 1-10 or 1-20 mol% CO. The microorganism of the disclosure typically converts at least a portion of the CO in the substrate to a product. In some embodiments, the substrate comprises no or substantially no (< 1 mol%) CO.

[0073] The substrate may comprise some amount of H₂. For example, the substrate may 20 comprise about 1, 2, 5, 10, 15, 20, or 30 mol% H₂. In some embodiments, the substrate may comprise a relatively high amount of H₂, such as about 60, 70, 80, or 90 mol% H₂. In further embodiments, the substrate comprises no or substantially no (< 1 mol%) H₂.

[0074] The substrate may comprise some amount of CO₂. For example, the substrate may comprise about 1-80 or 1-30 mol% CO₂. In some embodiments, the substrate may comprise 25 less than about 20, 15, 10, or 5 mol% CO₂. In another embodiment, the substrate comprises no or substantially no (< 1 mol%) CO₂.

[0075] Although the substrate is typically gaseous, the substrate may also be provided in alternative forms. For example, the substrate may be dissolved in a liquid saturated with a CO-containing gas using a microbubble dispersion generator. By way of further example, the 30 substrate may be adsorbed onto a solid support.

[0076] The substrate and/or C1-carbon source may be a waste gas obtained as a byproduct of an industrial process or from some other source, such as from automobile exhaust fumes,

electrolysis, pyrolysis, torrefaction, or gasification. For example, waste material may be recycled by pyrolysis, torrefaction, or gasification to generate the substrate and/or C1-carbon source. In certain embodiments, the industrial process is selected from the group consisting of ferrous metal products manufacturing, such as a steel mill manufacturing, non-ferrous products manufacturing, petroleum refining, coal gasification, electric power production, carbon black production, paper and pulp manufacturing, black liquor gasification, ammonia production, methanol production, and coke manufacturing. In these embodiments, the substrate and/or C1-carbon source may be captured from the industrial process before it is emitted into the atmosphere, using any convenient method.

- 10 **[0077]** The substrate and/or C1-carbon source may be syngas, such as syngas obtained by gasification of coal or refinery residues, gasification of biomass or lignocellulosic material, or reforming of natural gas. In another embodiment, the syngas may be obtained from the gasification of municipal solid waste or industrial solid waste.

- 15 **[0078]** The composition of the substrate may have a significant impact on the efficiency and/or cost of the reaction. For example, the presence of oxygen (O₂) may reduce the efficiency of an anaerobic fermentation process. Depending on the composition of the substrate, it may be desirable to treat, scrub, or filter the substrate to remove any undesired impurities, such as toxins, undesired components, or dust particles, and/or increase the concentration of desirable components.

- 20 **[0079]** In certain embodiments, the fermentation is performed in the absence of carbohydrate substrates, such as sugar, starch, lignin, cellulose, or hemicellulose.

- [0080]** The microorganism of the disclosure may be cultured with the gaseous substrate to produce one or more products. For instance, the microorganism of the disclosure may produce or may be engineered to produce ethanol (WO 2007/117157), acetate (WO 25 2007/117157), 1-butanol (WO 2008/115080, WO 2012/053905, and WO 2017/066498), butyrate (WO 2008/115080), 2,3-butanediol (WO 2009/151342 and WO 2016/094334), lactate (WO 2011/112103), butene (WO 2012/024522), butadiene (WO 2012/024522), methyl ethyl ketone (2-butanone) (WO 2012/024522 and WO 2013/185123), ethylene (WO 2012/026833), acetone (WO 2012/115527), isopropanol (WO 2012/115527), lipids (WO 30 2013/036147), 3-hydroxypropionate (3-HP) (WO 2013/180581), terpenes, including isoprene (WO 2013/180584), fatty acids (WO 2013/191567), 2-butanol (WO 2013/185123), 1,2-propanediol (WO 2014/036152), 1-propanol (WO 2017/066498), 1-hexanol (WO

2017/066498), 1-octanol (WO 2017/066498), chorismate-derived products (WO 2016/191625), 3-hydroxybutyrate (WO 2017/066498), 1,3-butanediol (WO 2017/066498), 2-hydroxyisobutyrate or 2-hydroxyisobutyric acid (WO 2017/066498), isobutylene (WO 2017/066498), adipic acid (WO 2017/066498), 1,3-hexanediol (WO 2017/066498), 3-methyl-
5 2-butanol (WO 2017/066498), 2-buten-1-ol (WO 2017/066498), isovalerate (WO 2017/066498), isoamyl alcohol (WO 2017/066498), and/or monoethylene glycol (WO 2019/126400) in addition to 2-phenylethanol. In certain embodiments, microbial biomass itself may be considered a product. These products may be further converted to produce at least one component of diesel, jet fuel, and/or gasoline. In certain embodiments, 2-
10 phenylethanol may be used as an ingredient in fragrances, essential oils, flavors, and soaps. Additionally, the microbial biomass may be further processed to produce a single cell protein (SCP).

[0081] A “native product” is a product produced by a genetically unmodified microorganism. For example, ethanol, acetate, and 2,3-butanediol are native products of *Clostridium*
15 *autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium ragsdalei*. A “non-native product” is a product that is produced by a genetically modified microorganism but is not produced by a genetically unmodified microorganism from which the genetically modified microorganism is derived.

[0082] “Selectivity” refers to the ratio of the production of a target product to the production
20 of all fermentation products produced by a microorganism. The microorganism of the disclosure may be engineered to produce products at a certain selectivity or at a minimum selectivity. In one embodiment, a target product account for at least about 5%, 10%, 15%, 20%, 30%, 50%, or 75% of all fermentation products produced by the microorganism of the disclosure. In one embodiment, the target product accounts for at least 10% of all
25 fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for the target product of at least 10%. In another embodiment, the target product accounts for at least 30% of all fermentation products produced by the microorganism of the disclosure, such that the microorganism of the disclosure has a selectivity for the target product of at least 30%.

30 **[0083]** “Increasing the efficiency,” “increased efficiency,” and the like include, but are not limited to, increasing growth rate, product production rate or volume, product volume per volume of substrate consumed, or product selectivity. Efficiency may be measured relative to

the performance of parental microorganism from which the microorganism of the disclosure is derived.

[0084] Typically, the culture is performed in a bioreactor. The term “bioreactor” includes a culture/fermentation device consisting of one or more vessels, towers, or piping
5 arrangements, such as a continuous stirred tank reactor (CSTR), immobilized cell reactor (ICR), trickle bed reactor (TBR), bubble column, gas lift fermenter, static mixer, or other vessel or other device suitable for gas-liquid contact. In some embodiments, the bioreactor may comprise a first growth reactor and a second culture/fermentation reactor. The substrate may be provided to one or both of these reactors. As used herein, the terms “culture” and
10 “fermentation” are used interchangeably. These terms encompass both the growth phase and product biosynthesis phase of the culture/fermentation process.

[0085] The culture is generally maintained in an aqueous culture medium that contains nutrients, vitamins, and/or minerals sufficient to permit growth of the microorganism. Preferably the aqueous culture medium is an anaerobic microbial growth medium, such as a
15 minimal anaerobic microbial growth medium. Suitable media are well known in the art.

[0086] The culture/fermentation should desirably be carried out under appropriate conditions for production of the target product. Typically, the culture/fermentation is performed under anaerobic conditions. Reaction conditions to consider include pressure (or partial pressure), temperature, gas flow rate, liquid flow rate, media pH, media redox potential, agitation rate
20 (if using a continuous stirred tank reactor), inoculum level, maximum gas substrate concentrations to ensure that gas in the liquid phase does not become limiting, and maximum product concentrations to avoid product inhibition. In particular, the rate of introduction of the substrate may be controlled to ensure that the concentration of gas in the liquid phase does not become limiting, since products may be consumed by the culture under gas-limited
25 conditions.

[0087] In certain embodiments, the fermentation is performed in the absence of light or in the presence of an amount of light insufficient to meet the energetic requirements of photosynthetic microorganisms. In certain embodiments, the microorganism of the disclosure is a non-photosynthetic microorganism.

30 [0088] In some embodiments, 2-phenylethanol may be separated or purified from a fermentation broth using any method or combination of methods known in the art, including, for example, fractional distillation, evaporation, pervaporation, gas stripping, phase

separation, and extractive fermentation, including for example, liquid-liquid extraction. In certain embodiments, 2-phenylethanol is recovered from the fermentation broth by continuously removing a portion of the broth from the bioreactor, separating microbial cells from the broth (conveniently by filtration), and recovering 2-phenylethanol from the broth.

- 5 Generally, alcohols and/or acetone may be recovered, for example, by distillation. Separated microbial cells are preferably recycled back to the bioreactor. The cell-free permeate remaining after 2-phenylethanol has been removed is also preferably returned to the bioreactor. Additional nutrients may be added to the cell-free permeate to replenish the medium before it is returned to the bioreactor.

10 **Embodiments**

[0089] In one embodiment, the disclosure provides a microorganism capable of producing 2-phenylethanol, wherein the microorganism comprises a heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde and a heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol.

- 15 **[0090]** In some embodiments, the heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde is decarboxylase and the heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol is phenylacetaldehyde reductase. In particular, the decarboxylase may be a phenylpyruvate-specific decarboxylase. In some embodiments, the nucleic acid encoding a heterologous decarboxylase enzyme comprises *aro10* (from
20 *Saccharomyces cerevisiae*) or *abPPDC* (from *Azospirillum brasilense*). In some embodiments, the nucleic acid encoding a heterologous decarboxylase enzyme comprises: (i) SEQ ID NO: 1, 2, or 4; (ii) a nucleic acid with at least 90% identity to SEQ ID NO: 1, 2, or 4 that encodes a decarboxylase enzyme that is functionally homologous to the decarboxylase enzyme encoded by SEQ ID NO: 1, 2, or 4; or (iii) a nucleic acid that encodes a functionally
25 homologous polypeptide with at least 90% identity to the decarboxylase enzyme encoded by SEQ ID NO: 1, 2, or 4.

- [0091]** In some embodiments, the nucleic acid encoding a heterologous decarboxylase enzyme comprises *aro10*, *abPPDC*, or SEQ ID NO: 1, 2, or 4, or a functional homologue thereof sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or
30 at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% nucleic acid sequence identity therewith. In

some embodiments, the nucleic acid encoding a heterologous decarboxylase enzyme encodes a functionally homologous polypeptide sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% polypeptide sequence identity to the decarboxylase enzyme encoded by *aro10*, *abPPDC*, or SEQ ID NO: 1, 2, or 4.

[0092] In some embodiments, the nucleic acid encoding a heterologous PAR enzyme comprises *blPAR*, *ecPAR*, *lePAR*, *rrPAR*, *rsPAR*, or *adh6* (source listed in Figure 4A). In some embodiments, the nucleic acid encoding a heterologous PAR enzyme comprises: (i) SEQ ID NO: 3, 5, 6, 7, 8, or 9; (ii) a nucleic acid with at least 90% identity to SEQ ID NO: 3, 5, 6, 7, 8, or 9 that encodes a PAR enzyme that is functionally homologous to the PAR enzyme encoded by SEQ ID NO: 3, 5, 6, 7, 8, or 9; or (iii) a nucleic acid that encodes a functionally homologous polypeptide with at least 90% identity to a PAR enzyme encoded by SEQ ID NO: 3, 5, 6, 7, 8, or 9.

[0093] In some embodiments, the nucleic acid encoding a heterologous PAR enzyme comprises *blPAR*, *ecPAR*, *lePAR*, *rrPAR*, *rsPAR*, *adh6*, or SEQ ID NO: 3, 5, 6, 7, 8, or 9, or a functional homologue thereof sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% nucleic acid sequence identity therewith. In some embodiments, the nucleic acid encoding a heterologous PAR enzyme encodes a functionally homologous polypeptide sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% polypeptide sequence identity to a PAR enzyme encoded by *blPAR*, *ecPAR*, *lePAR*, *rrPAR*, *rsPAR*, *adh6*, or SEQ ID NO: 3, 5, 6, 7, 8, or 9.

[0094] In some embodiments, the microorganism of the disclosure further comprises one or more of: a heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate; a heterologous enzyme that converts 3-dehydroshikimate to shikimate; or a heterologous enzyme that converts chorismate to phenylpyruvate.

[0095] In some embodiments, the heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase; the heterologous enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase; or the heterologous enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

[0096] In some embodiments, the nucleic acid encoding a heterologous shikimate dehydrogenase enzyme comprises *cgAroE* (from *Corynebacterium glutamicum*) or *ecAroE* (from *Escherichia coli*). In some embodiments, the nucleic acid encoding a heterologous shikimate dehydrogenase enzyme comprises: (i) SEQ ID NO: 11 or 12; (ii) a nucleic acid with at least 90% identity to SEQ ID NO: 11 or 12 that encodes a shikimate dehydrogenase enzyme that is functionally homologous to the shikimate dehydrogenase enzyme encoded by SEQ ID NO: 11 or 12; or (iii) a nucleic acid that encodes a functionally homologous polypeptide with at least 90% identity to the shikimate dehydrogenase enzyme encoded by SEQ ID NO: 11 or 12.

[0097] In some embodiments, the nucleic acid encoding a heterologous shikimate dehydrogenase enzyme comprises *cgAroE*, *ecAroE*, or SEQ ID NO: 11 or 12, or a functional homologue thereof sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% nucleic acid sequence identity therewith. In some embodiments, the nucleic acid encoding a heterologous shikimate dehydrogenase enzyme encodes a functionally homologous polypeptide sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% polypeptide sequence identity to a shikimate dehydrogenase enzyme encoded by *cgAroE*, *ecAroE*, or SEQ ID NO: 11 or 12.

[0098] In some embodiments, the nucleic acid encoding a heterologous DAHP synthase enzyme comprises *aroG*. In some embodiments, the nucleic acid encoding a heterologous DAHP synthase enzyme comprises: (i) SEQ ID NO: 10; (ii) a nucleic acid with at least 90% identity to SEQ ID NO: 10 that encodes a DAHP synthase enzyme that is functionally homologous to the DAHP synthase enzyme encoded by SEQ ID NO: 10; or (iii) a nucleic

acid that encodes a functionally homologous polypeptide with at least 90% identity to the DAHP synthase enzyme encoded by SEQ ID NO: 10.

- [0099]** In some embodiments, the nucleic acid encoding a heterologous DAHP synthase enzyme comprises *aroG* or SEQ ID NO: 10, or a functional homologue thereof sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% nucleic acid sequence identity therewith. In some embodiments, the nucleic acid encoding a heterologous DAHP synthase enzyme encodes a functionally homologous polypeptide sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% polypeptide sequence identity to a DAHP synthase enzyme encoded by *aroG* or SEQ ID NO: 10.
- [0100]** In some embodiments, the nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme comprises *pheA1* or *pheA2*. In some embodiments, the nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme comprises: (i) SEQ ID NO: 13 or 14; (ii) a nucleic acid with at least 90% identity to SEQ ID NO: 13 or 14, which encodes a bi-functional chorismate mutase/prephenate dehydratase enzyme that is functionally homologous to the bi-functional chorismate mutase/prephenate dehydratase enzyme encoded by SEQ ID NO: 13 or 14; or (iii) a nucleic acid that encodes a functionally homologous polypeptide with at least 90% identity to the bi-functional chorismate mutase/prephenate dehydratase enzyme encoded by SEQ ID NO: 13 or 14.
- [0101]** In some embodiments, the nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme comprises *pheA1*, *pheA2*, or SEQ ID NO: 13 or 14, or a functional homologue thereof sharing, for example, at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% nucleic acid sequence identity therewith. In some embodiments, the nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme encodes a functionally homologous polypeptide sharing, for example, at least 70%, or at least 75%, or

at least 80%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%,
 or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least
 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100% polypeptide
 sequence identity to a bi-functional chorismate mutase/prephenate dehydratase enzyme
 5 encoded by *pheA1*, *pheA2*, or SEQ ID NO: 13 or 14.

[0102] In some embodiments, the microorganism comprises a set of heterologous enzymes
 selected from among the following options:

- (1) ecAroE, kivD, lePAR, *pheA2*, and *aroG*;
- (2) ecAroE, abPPDC, ecPAR, *pheA1*, and *aroG*;
- 10 (3) cgAroE, abPPDC, lePAR, *pheA2*, and *aroG*;
- (4) ecAroE, abPPDC, lePAR, *pheA2*, and *aroG*;
- (5) cgAroE, kivD, rrPAR, *pheA2*, and *aroG*;
- (6) ecAroE, abPPDC, lePAR, *pheA2*, and *aroG*;
- (7) *pheA1*, *aroG*, abPPDC, and ecPAR;
- 15 (8) egAroE, abPPDC, lePAR, *pheA1*, and *aroG*;
- (9) ecAroE, abPPDC, ecPAR, *pheA2*, and *aroG*;
- (10) cgAroE, *aro10*, ecPAR, *pheA2*, and *aroG*;
- (11) cgAroE, abPPDC, lePAR, *pheA1*, and *aroG*;
- (12) cgAroE, *aro10*, lePAR, *pheA2*, and *aroG*;
- 20 (13) ecAroE, abPPDC, lePAR, *pheA1*, and *aroG*;
- (14) ecAroE, *aro10*, ecPAR, *pheA2*, and *aroG*;
- (15) ecAroE, *aro10*, lePAR, *pheA2*, and *aroG*;
- (16) cgAroE, abPPDC, lePAR, *pheA1*, and *aroG*;
- (17) cgAroE, *aro10*, rsPAR, *pheA2*, and *aroG*;
- 25 (18) cgAroE, abPPDC, blPAR, *pheA2*, and *aroG*;
- (19) ecAroE, kivD, blPAR, *pheA2*, and *aroG*;
- (20) cgAroE, *aro10*, blPAR, *pheA2*, and *aroG*;
- (21) ecAroE, abPPDC, blPAR, *pheA2*, and *aroG*;
- (22) ecAroE, abPPDC, *adh6*, *pheA1*, and *aroG*;
- 30 (23) cgAroE, abPPDC, *adh6*, *pheA2*, and *aroG*;
- (24) cgAroE, abPPDC, ecPAR, *pheA2*, and *aroG*;
- (25) ecAroE, *aro10*, rsPAR, *pheA2*, and *aroG*;
- (26) ecAroE, *aro10*, *adh6*, *pheA2*, and *aroG*; or

(27) *ecAroE*, *abPPDC*, *ecPAR*, *pheA2*, and *aroG*.

[0103] In some embodiments, the microorganism comprises a set of heterologous nucleic acids selected from among the following options:

- (1) *ecAroE*, *kivD*, *lePAR*, *pheA2*, and *aroG*;
- 5 (2) *ecAroE*, *abPPDC*, *ecPAR*, *pheA1*, and *aroG*;
- (3) *cgAroE*, *abPPDC*, *lePAR*, *pheA2*, and *aroG*;
- (4) *ecAroE*, *abPPDC*, *lePAR*, *pheA2*, and *aroG*;
- (5) *cgAroE*, *kivD*, *rrPAR*, *pheA2*, and *aroG*;
- (6) *ecAroE*, *abPPDC*, *lePAR*, *pheA2*, and *aroG*;
- 10 (7) *pheA1*, *aroG*, *abPPDC*, and *ecPAR*;
- (8) *egAroE*, *abPPDC*, *lePAR*, *pheA1*, and *aroG*;
- (9) *ecAroE*, *abPPDC*, *ecPAR*, *pheA2*, and *aroG*;
- (10) *cgAroE*, *aroI0*, *ecPAR*, *pheA2*, and *aroG*;
- (11) *cgAroE*, *abPPDC*, *lePAR*, *pheA1*, and *aroG*;
- 15 (12) *cgAroE*, *aroI0*, *lePAR*, *pheA2*, and *aroG*;
- (13) *ecAroE*, *abPPDC*, *lePAR*, *pheA1*, and *aroG*;
- (14) *ecAroE*, *aroI0*, *ecPAR*, *pheA2*, and *aroG*;
- (15) *ecAroE*, *aroI0*, *lePAR*, *pheA2*, and *aroG*;
- (16) *cgAroE*, *abPPDC*, *lePAR*, *pheA1*, and *aroG*;
- 20 (17) *cgAroE*, *aroI0*, *rsPAR*, *pheA2*, and *aroG*;
- (18) *cgAroE*, *abPPDC*, *blPAR*, *pheA2*, and *aroG*;
- (19) *ecAroE*, *kivD*, *blPAR*, *pheA2*, and *aroG*;
- (20) *cgAroE*, *aroI0*, *blPAR*, *pheA2*, and *aroG*;
- (21) *ecAroE*, *abPPDC*, *blPAR*, *pheA2*, and *aroG*;
- 25 (22) *ecAroE*, *abPPDC*, *adh6*, *pheA1*, and *aroG*;
- (23) *cgAroE*, *abPPDC*, *adh6*, *pheA2*, and *aroG*;
- (24) *cgAroE*, *abPPDC*, *ecPAR*, *pheA2*, and *aroG*;
- (25) *ecAroE*, *aroI0*, *rsPAR*, *pheA2*, and *aroG*;
- (26) *ecAroE*, *aroI0*, *adh6*, *pheA2*, and *aroG*; or
- 30 (27) *ecAroE*, *abPPDC*, *ecPAR*, *pheA2*, and *aroG*.

[0104] In some embodiments, the microorganism comprises the heterologous genes and promoters of combinatorial strain 2C03, 1C10, 2C54, 2C09, 2C47, 2C55, 2C53, H57, 1C47,

2C10, 2A18, 1C54, 2C01, 1C31, 2B17, 2C52, 1C09, H18, H58, 2C27, 2D22, 2D07, 2D24, 2D03, 1C29, 2B26, 2C38, 2A27, 2C12, or 2B27, as described in Table 3.

[0105] In another embodiment, the disclosure provides plasmids that can be used to transform bacteria to produce the 2-phenylethanol-producing bacteria of the disclosure. In some embodiments, the plasmids comprise (a) a nucleic acid encoding a heterologous decarboxylase enzyme; and (b) a nucleic acid encoding a heterologous phenylacetaldehyde reductase (PAR) enzyme. In some embodiments the plasmids comprise (a) a nucleic acid encoding a heterologous decarboxylase enzyme; (b) a nucleic acid encoding a heterologous phenylacetaldehyde reductase (PAR) enzyme; (c) a nucleic acid encoding a heterologous shikimate dehydrogenase enzyme; (d) a nucleic acid encoding a heterologous DAHP synthase enzyme; and (e) a nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme. In some embodiments, the disclosure provides a two-plasmid system, the first plasmid comprising (a) a nucleic acid encoding a heterologous decarboxylase enzyme; (b) a nucleic acid encoding a heterologous phenylacetaldehyde reductase (PAR) enzyme; and (c) a nucleic acid encoding a heterologous shikimate dehydrogenase enzyme; and a second plasmid comprising (d) a nucleic acid encoding a heterologous DAHP synthase enzyme; and (e) a nucleic acid encoding a heterologous bi-functional chorismate mutase/prephenate dehydratase enzyme. When the plasmid or plasmids are transformed into the bacteria, desirably the bacteria express the enzymes. Alternatively, the enzymes can be expressed only when induced, if an inducible promoter is used for expression.

[0106] One embodiment is a microorganism capable of producing 2-phenylethanol, wherein the microorganism comprises: (a) a heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde; and (b) a heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol.

[0107] The microorganism of an embodiment, wherein: (a) the heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde is decarboxylase; and (b) the heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol is phenylacetaldehyde reductase.

[0108] The microorganism of an embodiment, wherein the decarboxylase is a phenylpyruvate-specific decarboxylase.

- [0109] The microorganism of an embodiment, wherein the microorganism is a C1-fixing microorganism.
- [0110] The microorganism of an embodiment, wherein the microorganism is a Wood-Ljungdahl microorganism.
- 5 [0111] The microorganism of an embodiment, wherein the microorganism is a bacterium.
- [0112] The microorganism of an embodiment, wherein the microorganism is a member of a genus selected from the group consisting of Acetobacterium, Alkalibaculum, Blautia, Butyrivacterium, Clostridium, Eubacterium, Moorella, Oxobacter, Sporomusa, and Thermoanaerobacter.
- 10 [0113] The microorganism of an embodiment, wherein the microorganism is natively capable of producing phenylpyruvate.
- [0114] The microorganism of an embodiment, wherein the microorganism comprises a Wood-Ljungdahl pathway that converts CO, CO₂, and/or H₂ to acetyl-CoA.
- [0115] The microorganism of an embodiment, wherein the microorganism comprises one or
 15 more of: (c) a native enzyme that converts acetyl-CoA to pyruvate; (d) a native enzyme that converts pyruvate to phosphoenolpyruvate; (e) a native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate; (f) a native enzyme that converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquininate; (g) a native enzyme that converts 3-dehydroquininate to 3-
 20 dehydroshikimate; (h) a native enzyme that converts 3-dehydroshikimate to shikimate; (i) a native enzyme that converts shikimate to shikimate 3-phosphate; (j) a native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate; (k) a native enzyme that converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate; or (l) a native enzyme that converts chorismate to phenylpyruvate.
- 25 [0116] The microorganism of an embodiment, wherein: (c) the native enzyme that converts acetyl-CoA to pyruvate is pyruvate:ferredoxin oxidoreductase; (d) the native enzyme that converts pyruvate to phosphoenolpyruvate is pyruvate phosphate dikinase; (e) the native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate
 30 synthase; (f) the native enzyme that converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquininate is 3-dehydroquininate synthase; (g) the native enzyme that converts 3-dehydroquininate to 3-dehydroshikimate is 3-dehydroquininate dehydratase; (h) the

native enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase; (i) the native enzyme that converts shikimate to shikimate 3-phosphate is shikimate kinase; (j) the native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate is 5-enolpyruvylshikimate 3-phosphate synthase; (k) the native enzyme that
 5 converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate is chorismate synthase; or (l) the native enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

[0117] The microorganism of an embodiment, wherein the microorganism further comprises one or more of: (e) a heterologous enzyme that converts phosphoenolpyruvate and erythrose-
 10 4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate; (h) a heterologous enzyme that converts 3-dehydroshikimate to shikimate; or (l) a heterologous enzyme that converts chorismate to phenylpyruvate.

[0118] The microorganism of an embodiment, wherein: (e) the heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-
 15 heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase; (h) the heterologous enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase; or (l) the heterologous enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

[0119] The microorganism of any one of the embodiments, wherein the microorganism
 20 comprises:

- (1) ecAroE, kivD, lePAR, pheA2, and aroG;
- (2) ecAroE, abPPDC, ecPAR, pheA1, and aroG;
- (3) cgAroE, abPPDC, lePAR, pheA2, and aroG;
- (4) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- 25 (5) cgAroE, kivD, rrPAR, pheA2, and aroG;
- (6) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- (7) pheA1, aroG, abPPDC, and ecPAR;
- (8) egAroE, abPPDC, lePAR, pheA1, and aroG;
- (9) ecAroE, abPPDC, ecPAR, pheA2, and aroG;
- 30 (10) cgAroE, aro10, ecPAR, pheA2, and aroG;

- (11) cgAroE, abPPDC, lePAR, pheA1, and aroG;
- (12) cgAroE, aro10, lePAR, pheA2, and aroG;
- (13) ecAroE, abPPDC, lePAR, pheA1, and aroG;
- (14) ecAroE, aro10, ecPAR, pheA2, and aroG;
- 5 (15) ecAroE, aro10, lePAR, pheA2, and aroG;
- (16) cgAroE, abPPDC, lePAR, pheA1, and aroG;
- (17) cgAroE, aro10, rsPAR, pheA2, and aroG;
- (18) cgAroE, abPPDC, blPAR, pheA2, and aroG;
- (19) ecAroE, kivD, blPAR, pheA2, and aroG;
- 10 (20) cgAroE, aro10, blPAR, pheA2, and aroG;
- (21) ecAroE, abPPDC, blPAR, pheA2, and aroG;
- (22) ecAroE, abPPDC, adh6, pheA1, and aroG;
- (23) cgAroE, abPPDC, adh6, pheA2, and aroG;
- (24) cgAroE, abPPDC, ecPAR, pheA2, and aroG;
- 15 (25) ecAroE, aro10, rsPAR, pheA2, and aroG;
- (26) ecAroE, aro10, adh6, pheA2, and aroG; or
- (27) ecAroE, abPPDC, ecPAR, pheA2, and aroG.

[0120] The microorganism of any one of the embodiments, wherein the microorganism comprises the heterologous genes and promoters of combinatorial strain 2C03, 1C10, 2C54, 20 2C09, 2C47, 2C55, 2C53, H57, 1C47, 2C10, 2A18, 1C54, 2C01, 1C31, 2B17, 2C52, 1C09, H18, H58, 2C27, 2D22, 2D07, 2D24, 2D03, 1C29, 2B26, 2C38, 2A27, 2C12, or 2B27.

[0121] The microorganism of any one of the embodiments, wherein the microorganism ferments a gaseous substrate comprising CO, CO₂, and/or H₂ to produce 2-phenylethanol.

[0122] The microorganism of an embodiment, wherein the gaseous substrate comprises 25 syngas or industrial waste gas.

[0123] The microorganism of an embodiment, wherein the microorganism does not produce any other C₃+ alcohols.

[0124] The microorganism of an embodiment, wherein the microorganism does not produce any other C3, C4, C5, C6, C7, C8, C9, or C10 alcohols.

[0125] One embodiment is a method of producing 2-phenylethanol comprising culturing the microorganism of any one of the embodiments in the presence of a gaseous substrate.

- 5 [0126] The method of an embodiment, wherein the gaseous substrate comprises a C1-carbon source comprising CO, CO₂, and/or H₂.

[0127] The method of any one of the embodiments, wherein the gaseous substrate comprises syngas or industrial waste gas.

10 EXAMPLES

[0128] The following examples further illustrate the methods and compositions of the disclosure but should not be construed to limit its scope in any way.

- [0129] In this work, targeted metabolic engineering of the decarboxylase and phenylacetaldehyde reductase (PAR) was carried out in C1-fixing bacteria to improve 2-PE selectivity and production. To further improve the flux towards 2-PE biosynthesis, three
15 heterologous genes from the shikimate pathway were additionally included in a combinatorial manner. The 2-PE biosynthesis pathway and the genes that were heterologously expressed in the following Examples are highlighted in Figure 1.

20 *Example 1. 2-PE Tolerance of C. autoethanogenum in Schott Bottles*

- [0130] 2-PE has been reported to exert a growth inhibitory effect on Gram-positive microorganisms such as *Staphylococcus aureus* and *Enterococcus faecium* at 26 – 43 mM (3.2 – 5.3 g/L) levels (Corre et al., Res Microbiol. 1990;141(4):483-97). To study the toxicity of 2-PE on autotrophic growth of *C. autoethanogenum*, a 2-PE challenge experiment (at 0,
25 0.2, 0.6, 2 and 5 g/L) was conducted in Schott bottles with synthetic gas blend (50% CO, 10% H₂, 30% CO₂ and 10% N₂). In comparison to the unchallenged culture, 2 g/L of 2-PE resulted in an increase in growth lag phase and lower biomass concentrations (Figures 2A-B). At 5 g/L challenge level, no growth was detected (Figures 2A-B). 2-PE challenge at 0.2 g/L and 0.6 g/L had little impact on autotrophic growth of *C. autoethanogenum*.

30

Example 2. Testing of Decarboxylase Variants for Improved 2-PE Selectivity and Production

- [0131] For 2-PE strain optimization, two plasmids with phenylpyruvate-specific decarboxylases (*aro10* from *Saccharomyces cerevisiae* (Kneen et al., FEBS J. 2011;278:1842–53) (SEQ ID NO: 4) and *abPPDC* from *Azospirillum brasilense* (Spaepen et al., J Bacteriol. 2007;189:7626 LP – 7633) (SEQ ID NO: 2)) were transformed into *C. autoethanogenum*, resulting in strains sFA212 and sFA213, respectively. These two decarboxylase strains, together with a control strain (sFA200) that expresses decarboxylase *kivd_la* (from *Lactococcus lactis*) (SEQ ID NO: 1), were subjected to autotrophic growth under synthetic gas mix (50% CO, 10% H₂, 30% CO₂, and 10% N₂) in Schott bottles (Figures 3 A-B). The three decarboxylase strains displayed similar growth profiles but different alcohol profiles (Figures 3 A-B). End-point gas chromatography with flame ionization detection (GC-FID) results showed that the *aro10* strain produced 57% less 2-PE than the control *kivd_la* strain, while producing 5.6 mg/L 2-methyl-1-propanol and 4.0 mg/L 3-methyl-1-butanol. In contrast, the *abPPDC* strain produced similar amounts of 2-PE as the control strain, but none of the branch-chain alcohols. In some embodiments, the increased selectivity towards 2-PE by the *abPPDC* strain could simplify downstream product separation and improve strain stability as branch-chain alcohols could exert an additive effect on cell toxicity.
- [0132] For this and the other examples disclosed herein, GC-FID was performed for linear and branched-chain alcohols as follows. Alcohol concentrations were measured by gas chromatography analysis using an Agilent 7890B GC equipped with an autosampler, flame ionization detection (FID) and a Phenomenex ZB-WAXplus column (30m x 0.32 mm x 1 µm) linked in series to a ZB-1 column (30 m x 0.32 mm x 0.3 µm). Samples were prepared by transferring 1.400 mL of sample to a clean 2-mL microcentrifuge tube followed by the addition of 20 µL of an internal standard solution (phenyl acetate in ethanol). To this, 400 µL of chloroform was added and the mass recorded. Samples were shaken horizontally for 60 seconds, centrifuged at 14,000 x g for 5 minutes, then 200 µL of the bottom layer was transferred to a glass vial containing a low-volume insert. Analysis of a 1-µL injection was performed using a split ratio of 10 to 1 and an inlet temperature of 250 °C. Separation was achieved with a starting oven temperature of 70 °C (no hold), an initial ramp to 120 °C at 3 °C/min (no hold), and a final ramp to 230 °C at 7 °C/min (14 minute hold). Column flow rate was set to 35 cm/sec with helium as the carrier gas. The FID was set to 280 °C with air at 400

mL/min, hydrogen gas at 40 mL/min, and helium (makeup gas) at 15 mL/min. Results were calculated using an internal standard calibration using a linear fit.

Example 3. Testing of Phenylacetaldehyde Reductase (PAR) Variants for Improved 2-PE Production

[0133] Five gene variants (*lePAR* (SEQ ID NO: 9), *ecPAR* (SEQ ID NO: 6), *blPAR* (SEQ ID NO: 5), *rrPAR* (SEQ ID NO: 7), and *rsPAR* (SEQ ID NO: 8); Figures 4A-B) of phenylacetaldehyde reductase (PAR), which catalyzes the last step of the 2-PE pathway (Figure 1), were individually cloned into expression vectors with decarboxylase *abPPDC* (SEQ ID NO: 2) and shikimate dehydrogenase *ecAroE* (SEQ ID NO: 11). Following transformation into *C. autoethanogenum*, the resulting recombinant strains were tested for 2-PE production in 12-well plates with 200 kPa synthetic gas mix (50% CO, 10% H₂, 30% CO₂, and 10% N₂). Inducer was added on day 1, and samples were collected for GC-FID analysis on day 7. Three of the new *PAR* strains (*blPAR*, *ecPAR*, and *rsPAR*) produced more 2-PE (normalized by biomass) than the *adh6* (SEQ ID NO: 3) control strain (Figures 4C).

Example 4. Combinatorial Analysis of 2-PE Pathway

[0134] In order to determine the optimum flux and gene variants for the biosynthesis of 2-PE, a combinatorial assembly of the 2-PE pathway genes and promoters using the Golden Gate (GG) method was first performed in *Escherichia coli* (Figure 5) before transformation into C1-utilizing microorganisms. To facilitate the screening of assembly, a *ccdB* toxin-antitoxin counter-selectable marker flanked by Golden Gate site GG1 and GG6 was first cloned into *Clostridium-E. coli* shuttle vector pMTL8225 (Heap, J Microbiol Methods, 78: 79-85, 2009). These shuttle vectors have a pre-cloned clostridial promoter (shown as P₁ in Figure 5) and terminator (shown as T₃ in Figure 5). The genes that encode shikimate dehydrogenase (encoded by *aroE*), DAHP synthase (encoded by *aroG*), bi-functional chorismate mutase/prephenate dehydratase (*pheA*), decarboxylase, and phenyl-acetaldehyde (PAR), together with two donor vectors (pDonor 2 and pDonor 4, which provide the terminators and promoters), were added to the shuttle vectors for GG assembly. The promoter sequences, Golden Gate sites, and assembly workflow are described in Synthetic Biology (2020) vol 5(1): ysaa019. The resulting combinatorial plasmids with *ermB* antibiotic selectable marker

(collectively called plasmid 1 in Table 3) have a promoter and terminator to express each of *aroE* (or *pheA+aroG*), decarboxylase and PAR in an insulated manner.

[0135] For this work, two 2-PE combinatorial libraries were constructed and tested: one-plasmid and two-plasmid libraries (Table 3). The gene variants (2x *aroE* (SEQ ID NOs: 11, 12), 3x decarboxylase (SEQ ID NOs: 1, 2, 4), 6x PAR (SEQ ID NOs: 3, 5, 6, 7, 8, 9), 2x bi-functional chorismate mutase/prephenate dehydratase (*pheA*) (SEQ ID NOs: 13, 14), and 1x DAHP synthase (*aroG*) (SEQ ID NO: 10)) that were included in the combinatorial analysis are shown in Table 2. (Figure 12) shows gene and promoter variants that were employed for the two 2-PE combinatorial libraries described in Example 4.

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Table 2. 2-PE pathway gene variants employed for combinatorial analysis.

Enzyme	Gene [SEQ ID NO]	Description	Reference
DAHP synthase (EC 2.5.1.54)	<i>aroG</i> [10]	<i>E. coli</i> ; L175D to alleviate phenylalanine feedback regulation	Hu et al., J Basic Microbiol. 2003;43(5):399-406.
Shikimate dehydrogenase (EC 1.1.1.25)	<i>cgAroE</i> [12]	<i>Corynebacterium glutamicum</i>	Kubota et al., Appl Microbiol Biotechnol. 2013;97:8139-49.
	<i>ecAroE</i> [11]	<i>E. coli</i>	Sun et al., Appl Environ Microbiol. 2013; 79(13):4024-30.
Chorismate mutase/prephenate dehydratase (EC 4.2.1.51)	<i>pheA1</i> [13]	<i>E. coli</i> ; Deletion of 49 residues at 3' end to alleviate phenylalanine feedback regulation	US 4,753,883
	<i>pheA2</i> [14]	<i>E. coli</i> ; W338R to alleviate phenylalanine feedback regulation	US 4,753,883
Decarboxylase (EC 4.1.1.74)	<i>abPPDC</i> [2]	<i>Azospirillum brasilense</i>	Spaepen et al., J Bacteriol. 2007;189:7626 LP – 7633.
	<i>aro10</i> [4]	<i>Saccharomyces cerevisiae</i>	Kneen et al., FEBS J. 2011;278:1842-53.
	<i>kivD_la</i> [1]	<i>Lactococcus lactis</i>	De La Plaza et al., FEMS Microbiol Lett. 2004; 238(2):367-74.
Phenylacetaldehyde reductase (EC 1.1.1.1)	<i>blPAR</i> [5]	<i>Brevibacterium linens</i>	Hirano et al., Appl Microbiol Biotechnol. 2007;76(2):357-63.
	<i>ecPAR</i> [6]	<i>E. coli</i>	Guo et al., Microbiologyopen. 2017 Aug;6(4).
	<i>lePAR</i> [9]	<i>Solanum lycopersicum</i> (tomato)	Tieman et al., Phytochemistry. 2007;68(21):2660-9.

	<i>rrPAR</i> [7]	<i>Rhodococcus ruber</i>	Giersberg et al., J Ind Microbiol Biotechnol. 2012 Sep;39(9):1385-96.
	<i>rsPAR</i> [8]	<i>Rhodococcus</i> sp. ST-10	Itoh et al., Appl Environ Microbiol. 1997 Oct;63(10):3783-8.
	<i>adh6</i> [3]	<i>S. cerevisiae</i>	Larroy et al., Chem Biol Interact. 2003 Feb 1;143-144:229-38.

[0136] In the case of the two-plasmid library, three promoters of different strengths ($P_{\text{fer-lacO-US}}$, P_{WL} and P_{pfor}) were used to express each of *aroE*, decarboxylase, and PAR in plasmid 1. Plasmid 2 (catP antibiotic selectable marker), which had only one promoter (P_{WL}) that drove the expression of *pheA1+aroG* or *pheA2+aroG*, was individually transformed into *C. autoethanogenum*. The resulting transformants subsequently became the host into which the combinatorial plasmid 1 was transferred, resulting in recombinant strains that carried two expression vectors conferring different antibiotic resistance with compatible Gram-positive replicons. The total permutation for the two-plasmid combinatorial library was 1944 (3 x 2 x 3 x 3 x 3 x 6 x 1 x 2 x 1).

[0137] In the case of the one-plasmid library, the *aroE* in plasmid 1 was replaced by (*pheA1/pheA2*) + *aroG*. This resulted in only 1 plasmid (with *aroE* omitted) and a permutation of 972 (3 x 2 x 3 x 3 x 3 x 6). During this process, the spacer distance between the ribosomal binding site and START codon of *aroG* was extended from 5 to 8 nucleotides to enhance the translation of *aroG*.

[0138] To determine the assembly efficiency of combinatorial plasmids in *E. coli*, and to investigate the promoter and gene variant combinations, 400 plasmids were extracted from transformed *E. coli* strain NEB10-beta and subjected to sequencing. Analysis of the sequencing results showed an assembly rate of 51.3%, with good diversity of each promoter and gene variants. Following transformation of these sequence-verified combinatorial plasmids into *C. autoethanogenum*, a total of 162 combinatorial strains (42 one-plasmid strains and 120 two-plasmid strains) were subjected to autotrophic growth in 12-well plates.

[0139] Growth experiments were conducted with technical duplicates in 12-well plates with 2 mL minimal media and 200 kPa of synthetic gas mix (50% CO, 10% H₂, 30% CO₂, and 10% N₂) at 37 °C for 8-10 days (Figures 7A-C). Broth samples were then taken for biomass measurement and GC-FID analysis to determine 2-PE titer. Three biological clones of strain

sFA212, which harbors a plasmid (of the same backbone as plasmid 1) that expresses *adh6* with *abPPDC* under the inducible promoter *P_{ip112}*, were chosen as a control strain because this strain consistently produced high amounts of 2-PE in 12-well plates, Schott bottles, and continuously stirred tank reactors (CSTRs). One media blank control well per 12-well plate was included as negative control and GC-FID analysis showed <1.7 mg/L 2-PE, which indicates little-to-no cross-well contamination.

[0140] Following 8-10 days of incubation, the 2-PE combinatorial strains reached biomass concentration of 0.6 – 1.0 gDCW/L (Figures 7C). Out of these 162 combinatorial strains, 80 strains (49% of the screened library) produced >1.5-fold more 2-PE than the control strain (Figures 7A). 48 strains (30% of the screened library) produced less than half of the 2-PE produced by the control strain (Figures 7A).

[0141] Detailed analysis of the 2-PE combinatorial library was conducted to discern the effects of gene variants and number of plasmids on 2-PE production (Figures 8A-E). No significant difference in 2-PE titer was observed between the one-plasmid and two-plasmid libraries, decarboxylases, and shikimate dehydrogenases (Figures 8A, 8C, and 8D). Strains with *pheA2* showed slightly higher 2-PE titers than strains with *pheA1* (Figures 8B). Some of the PAR variants (e.g., *bIPAR*) produced more 2-PE than the *adh6* variant (Figures 8E). The large 2-PE titer variations observed in each gene variant or plasmid number could be attributed to the collective effect of other pathway gene variants and promoter combinations, which diluted the effect of single parameters in the combinatorial design.

[0142] By ranking the 2-PE titer relative to the control strain sFA212, a list of top 30 2-PE producing combinatorial strains with their genotype was produced (Table 3). Although 74% of the screened combinatorial library were two-plasmid strains with either *aroE*, 90% of the top 30 2-PE producing strains carried two plasmids. All top 10 2-PE producing strains consisted of either decarboxylase variant *kivD_1a* or *abPPDC*, whereas strains with decarboxylase *aro10* appeared 8 times between the top 11 and top 30 2-PE producers. Strains with PAR variant *lePAR* and *ecPAR* accounted for 70% of the top 30 2-PE producers, disproportionately high relative to their overall representation of 57% in the screened library. Strains with PAR variant *adh6* were significantly under-represented in the top 30 2-PE producers: only 6.7% in the top 30 vs. 13.6% in the screened library overall.

Table 3. Genotype of top 30 2-PE producing combinatorial strains in the 12-well plate growth assay.

Strain Name	Average 2PE titer relative to sFA212	Plasmid 1					Plasmid 2			
		P1	Gene 1	P2	Gene 2	P3	Gene 3	P4	Gene 4	Gene 5
2C03	3.01	Ppfor	<i>ecAroE</i>	Pwl	<i>kivD</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
1C10	2.99	Ppfor	<i>ecAroE</i>	Pwl	<i>abPPDC</i>	Pfer	<i>ecPAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
2C54	2.94	Ppfor	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C09	2.92	Ppfor	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pfer	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C47	2.91	Ppfor	<i>ecAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C55	2.85	Ppfor	<i>cgAroE</i>	Pwl	<i>kivD</i>	Pwl	<i>rrPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C53	2.75	Ppfor	<i>ecAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
H57	2.72	Pwl	<i>pheA1 + aroG</i>	Ppfor	<i>abPPDC</i>	Pfer	<i>ecPAR</i>	None		
1C47	2.71	Ppfor	<i>ecAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
2C10	2.70	Ppfor	<i>ecAroE</i>	Pwl	<i>abPPDC</i>	Pfer	<i>ecPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2A18	2.69	Pfer	<i>cgAroE</i>	Pwl	<i>aro10</i>	Ppfor	<i>ecPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
1C54	2.66	Ppfor	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
2C01	2.63	Ppfor	<i>cgAroE</i>	Pfer	<i>aro10</i>	Pfer	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
1C31	2.62	Ppfor	<i>ecAroE</i>	Pfer	<i>abPPDC</i>	Pwl	<i>lePAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
2B17	2.54	Pwl	<i>ecAroE</i>	Ppfor	<i>aro10</i>	Ppfor	<i>ecPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C52	2.52	Ppfor	<i>ecAroE</i>	Pfer	<i>aro10</i>	Ppfor	<i>lePAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
1C09	2.48	Ppfor	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pfer	<i>lePAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
H18	2.48	Pwl	<i>pheA1 + aroG</i>	Pfer	<i>abPPDC</i>	Ppfor	<i>ecPAR</i>	None		
H58	2.43	Ppfor	<i>pheA1 + aroG</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>ecPAR</i>	None		
2C27	2.39	Ppfor	<i>cgAroE</i>	Pwl	<i>aro10</i>	Ppfor	<i>rsPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2D22	2.35	Pfer	<i>cgAroE</i>	Pfer	<i>abPPDC</i>	Pfer	<i>blPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2D07	2.33	Pfer	<i>ecAroE</i>	Pwl	<i>kivD</i>	Pfer	<i>blPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2D24	2.33	Ppfor	<i>cgAroE</i>	Pwl	<i>aro10</i>	Ppfor	<i>blPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2D03	2.32	Pfer	<i>ecAroE</i>	Pfer	<i>abPPDC</i>	Pfer	<i>blPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
1C29	2.29	Ppfor	<i>ecAroE</i>	Pfer	<i>abPPDC</i>	Pfer	<i>lePAR</i>	Pwl	<i>pheA1</i>	<i>aroG</i>
2B26	2.28	Pwl	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pfer	<i>adh6</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C38	2.27	Ppfor	<i>cgAroE</i>	Ppfor	<i>abPPDC</i>	Pfer	<i>ecPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2A27	2.26	Pfer	<i>ecAroE</i>	Pwl	<i>aro10</i>	Ppfor	<i>rsPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2C12	2.26	Ppfor	<i>ecAroE</i>	Pwl	<i>aro10</i>	Pfer	<i>Adh6</i>	Pwl	<i>pheA2</i>	<i>aroG</i>
2B27	2.24	Pwl	<i>ecAroE</i>	Ppfor	<i>abPPDC</i>	Pwl	<i>ecPAR</i>	Pwl	<i>pheA2</i>	<i>aroG</i>

5 Example 5. 2-PE Production from Syngas Fermentation in CSTRs

[0143] A total of 12 combinatorial strains were characterized in CSTR under continuous fermentation mode to compare their 2-PE production to reference strain sFA212. Actively growing (early exponential) culture from Schott bottles was used as inoculum for 2-L CSTRs with a synthetic gas blend (40% CO, 20% H₂, 20% CO₂, and 20% N₂) at atmospheric pressure. Once the biomass concentration reached ~0.5 gDCW/L, a media dilution rate of 1/day and a bacterial dilution rate of 0.5/day were maintained.

[0144] Under these continuous CSTR conditions, the reference strain sFA212 achieved a 2-PE titer of 70 mg/L. Five combinatorial strains (1C29, 2C54, 2C03, 2C53 and 2C31)

produced more 2-PE than the reference strain (Figures 9A). In particular, strain 1C29 produced 200 mg/L of 2-PE, 2.9-fold higher than the reference strain. Based on their 2-PE yield, 10 of the 12 combinatorial strains displayed superior 2-PE performance relative to the reference strain (Figures 9B).

- 5 [0145] The performance of combinatorial strain 1C29 in two replicate CSTR runs is shown in Figures 10A-B. To enhance 2-PE production, nitrogen supply in the form of NH_4OH was limited to 15 mM on day 7 for replicate 1 (Figure 10A), and day 9 for replicate 2 (Figure 10B). As a result, 2-PE production increased in both runs and peaked at 350 and 300 mg/L, respectively. The 2-PE productivity momentarily peaked at 16 mg/L/h for run 1.
- 10 [0146] Syngas derived from gasification of waste materials such as biomass, forestry residues, and municipal solid waste often contains toxic contaminants. For example, methane, ethane, ethylene, and acetylene are among the detected contaminants and they are known to be toxic to microbes at ppm levels. To assess the robustness of the *de novo* 2-PE biosynthesis in engineered *C. autoethanogenum*, strain sFA212 was subjected to continuous CSTR run
- 15 using syngas derived from corn-stover. This “real” syngas was pre-treated using a gas treatment system, resulting in a post-treatment gas composition of 23.3% H_2 , 38.7% CO , 19.6% CO_2 , and 18.4% N_2 . Due to the limited availability of the real syngas, synthetic blended gas (with similar gas composition) was used initially to establish a steady state fermentation culture before the gas supply was switched to real syngas on day 6.9.
- 20 [0147] The results of real syngas fermentation are shown in (Figures 11A-C), with real syngas being used between day 6.9 and 10.9 (total of 4 days). In order to enable longer continuous CSTR run, several real syngas bottles were used with slightly different gas compositions, which resulted in the observed gas uptake fluctuations (Figure 11B). Following the switch to real syngas, the 2-PE production continued to increase and reached a steady
- 25 state on day 10. The average titer and productivity of 2-PE between day 8.0 and day 13.7 is 208 mg/L and 11.95 mg/L/h, respectively.

SEQUENCES

Table 4. Sequences for the nucleic acids described in the Examples and summarized in Table 2.

SEQ ID NO	Description	Sequence
1	LZ1561 codon adapted nucleotide sequence of <i>kivD_la</i>	ATGTATACAGTTGGAGATTATTTATTAGATAGATTACATGAATTAGG AATAGAAGAAATATTTGGTGTACCAGGAGATTACAATTTACAATTTT TAGATCAAATAATAAGTAGAAAAGATATGAAATGGGTGGGAATGCA AATGAGTTAAATGCAAGTTACATGGCTGATGGATATGCAAGAATAA AAAGGCAGCTGCATTCTTACAACCTTTGGAGTAGGAGAACTAAGTG CAGTAAATGGGCTTGCAGGTTTCATATGCTGAAAACCTACCTGTAGTT GAAATCGTAGGTAGTCCAACCTTCAAAGTCCAAAACGAAGGAAAATT TGTACACCATACTCTGGCTGACGGAGATTTTAAACATTTTATGAAAA TGCATGAACCTGTAACAGCTGCGAGAACCCTTTTAACTGCGGAAAAAT GCTACAGTTGAAATAGATAGAGTTTAAAGTGCTCTTTTAAAGGAGAG AAAGCCTGTTTATATTAATCTTCCGTTAGATGTAGCTGCTGCTAAGG CAGAGAAACCTTCTTTACCTTTGAAAAAGGAAAACAGCACTTCTAAT ACTTCCGATCAAGAGATATTAAATAAAATTCAGAATCCTTAAAAAA TGCTAAAAAACCTATAGTTATTACAGGACACGAAATAATATCTTTTG GCCTAGAAAAACAGTAAGTCAGTTTATAAGTAAACAAAATTACCT ATAACCTACTTTAAATTTTCGGTAAGAGTTCTGTTGACGAGGCACTTCC AAGTTTTTTAGGAATATATAATGGAAAATTGAGTGAACCAATCTAA AAGAGTTTGTAGAGTCAGCAGACTTTATACTAATGCTTGGTGTGAAG TTAACCTGATTCAGTACTGGAGCCTTTACTCATCTTTAAATGAAAA CAAAATGATAAGTTTAAACATTTGATGAGGGCAAAATTTTCAATGAGT CAATTCAAAATTTTGATTTTGAATCTTTAATTTTCATCACTTCTGGAT TTATCAGAAATAGAGTATAAAGGCAATATATTGATAAGAAACAAGA AGATTTTGTTCGAAGTAATGCACTATTAAAGTCAAGATAGGTTATGGC AGGCAGTTGAAAATTTAACTCAAAGCAATGAGACTATAGTAGCAGAA CAGGGAACATCACTATTTGGAGCGTCTTCTATTTTCTTAAGCCAAA AAGTCATTTTATAGGTGAGCATTATGGGGTTCTATAGGATATACCTT TTCCAGCTGCATTAGGATCAGAGATAGCAGATAAAGAATCTAGACAT CTTTTGTTTCATAGGCGATGGTTCCTTGCAGTTAACAGTCCAGGAATT AGGACTTGCAATAAGAGAAAAAATAAACCTATTTGTTTCATAAATAA ATAATGATGGATATACTGCTGAAAGAGAAATACATGGACCAAATCAG AGCTATAATGATATTCCAATGTGGAATATTCTAACTGCCTGAATC ATTTGGGGCTACAGAAGAAAGGGTAGTGTCAAAAATAGTTAGAACAG AAAATGAATTTGTAAGTGTTCATGAAAGAAGCTCAGGCTGATCCAAAC AGAATGTATTGGATTGAACCTACTTGCAAAAGAGGATGCTCCAAA AGTATTAAAGAAAATGGGGAACTTTTTGCTGAACAAAACAAATCTT AA
2	LZ1561 codon adapted nucleotide sequence of <i>abPPDC</i>	ATGAAATTGGCAGAAGCATTACTCAGAGCATTGAAAGATAGAGGGGC ACAGGCAATGTTTGGTATACCTGGAGATTTGCACTTCCTTTTTTCA AAGTTGCTGAAGAACTCAAATACTTCTTTGCACACTCTTAGCCAT GAACCTGCTGTAGGTTTTTGCAGCAGATGCAGCTGCAAGATATTCTTC TACATTAGGTGTAGCAGCAGTTACTTACGGAGCAGGAGCATTTAATA TGGTAAATGCAGTTGCTGGTGCCTATGCAGAAAAAGCCCTGTAGTA GTTATATCTGGTGTCTCCTGGTACAACAGAGGAAATGCTGGTCTTTT ACTTCACCATCAGGGAAGAACTTTAGACACTCAATTCAGGTATTTA AGGAGATAACAGTTGCCCAGGCTAGATTAGATGATCCTGCTAAGGCT CCTGCCGAAATAGCAAGAGTACTTGGTGTGCTAGAGCGCTCTCAAG ACCAGTTTATTTAGAAATACCTAGAAATATGGTAAATGCTGAAGTAG AACCAGTAGGGGATGATCCAGCATGGCCAGTAGACAGAGATGCTTTA GCTGCCTGTGCTGATGAAGTACTTGCAGCTATGAGATCTGCTACAAG TCCTGTTTTAATGGTTTGTGTAGAGTTAGAAGATATGGACTTGAAG

		CAAAAGTTGCTGAATTAGCACAGAGACTTGGAGTACCTGTAGTAACA ACATTTATGGGACGCGGATTACTTGCAGATGCACCCACTCCTCCTTT AGGAACCTATATAGGAGTAGCAGGAGATGCAGAAATACTAGATTAG TGGAGAATCAGATGGATTATTTTACTTGGAGCAATTCTTTCAGAT ACAAATTTTGCAGTCTCTCAAAGAAAAATAGACTTAAGAAAAACCAT TCATGCATTTGATCGTGCAGTTACCCTTGGATATCATACTTATGCTG ACATTCCTTTAGCAGGACTAGTTGATGCCCTTCTAGAAAGACTTCCT CCTTCAGACAGGACGACTAGAGGTAAAGAGCCACATGCCTATCCTAC AGGTCTTCAGGCTGATGGAGAACCAATAGCTCCAATGGACATTGCC GTGCTGTGAACGACAGAGTACGTGCTGGTCAGGAACCTCTTTTAATA GCAGCTGATATGGGTGACTGCTTATTTACAGCTATGGATATGATAGA TGCTGGTCTTATGGCACC GGCTATTATGCAGGCATGGGATTGGAG TTCCAGCTGGTATTGGTGCTCAATGTGTATCAGGCGGCAAAAGAATA CTTACTGTTGTTGGTGACGGAGCATTCCAGATGACAGGCTGGGAAC TGGAATTTGTAGAAGATTAGGTATTGACCTATAGTAATACTTTTTTA ATAATGCTTCTTGGGAAATGTTAAGAACATTTTCAGCCTGAAAGTGCT TTTAATGATTTAGATGATTGGAGATTTGCAGATATGGCTGCTGGAAT GGGCGGCGACGGTGTAAAGAGTCAGGACAAGGGCTGAGTTGAAGGCTG CATTAGATAAAGCATTGCCCCAAGGGGCAGATTCCAGCTTATAGAA GCAATGATACCGAGAGGTGTGCTTTCCGATACCCTTGCTAGATTTGT TCAAGGCCAAAAGAGGCTTCACGCAGCTCCTAGAGAATAA
3	LZ1561 codon adapted nucleotide sequence of <i>adh6</i>	ATGTCGTATCCTGAAAAATTTGAAGGTATAGCTATTCAATCCCATGA AGACTGGAAAAATCCCAAAAAACAAAGTACGACCCAAACCTTTCT ATGACCATGATATAGATATAAGATTGAAGCATGTGGAGTATGTGGA AGTGATATTCATTGTGCGGCAGGCCATTGGGGAAATATGAAAATGCC ATTAGTTGTGGGACATGAAATTTGTTGGAAAAGTTGTAAGTTAGGTC CTAAATCAAATCTGGACTAAAAGTTGGACAGAGAGTTGGAGTTGGT GCTCAAGTATTTCTTGTTTAGAGTGTGATAGATGTAAAAATGACAA TGAACCTTACTGTACTAAATTTGTTACTACTTATTCCAAACCTTATG AAGATGGATATGTAAGTCAGGGAGGCTATGCAAACTATGTTAGAGTT CACGAGCACTTTGTAGTACCTATTCCAGAAAATATTCATCTCACTT AGCAGCTCCTCTTTTATGCGGTGGACTTACTGTTTATAGTCCAGCTG TTAGAAATGGTTGTGGTCTGGGAAAAAAGTCGGAATAGTTGGACTT GGCGGAATTGGAAGTATGGGAACCTTTAATAAGTAAAGCTATGGGAGC TGAACCTTATGTAATTTCTAGATCATCAAGAAAGAGAGAAGATGCAA TGAAGATGGGAGCAGATCACTATATTGCTACATTAGAAGAGGGGGAT TGGGGGGAAAAGTACTTTGATACTTTTGATTTAATTGTCGTATGTGC TTCAAGTCTTACAGATATAGATTTTAATATAATGCCAAAAGCAATGA AAGTTGGTGGACGAATAGTATCTATAAGTATACCTGAACAGCACGAA ATGTTATCTTTAAACCTTATGGACTAAAGGCTGTATCTATTTCTTA TTCTGCATTGGGCTCTATTAAAGAATTGAATCAATTATAAAACCTGG TTAGTGAAAAAGATATAAAAAATTTGGGTAGAAACACTTCCTGTAGGT GAGGCAGGAGTCCATGAGGCTTTTGAGAGAATGGAAAAAGGTGATGT AAGATATAGATTTACACTTGTGGCTATGATAAAGAGTTTCTGATT AG
4	LZ1561 codon adapted nucleotide sequence <i>aro10</i>	ATGGCACCAGTAACAATTGAAAAATTTGTAAATCAAGAGGAAAGACA TTTGGTATCAAACCGTTTCAGCTACAATTCCATTTGGGGAAATATATAT TCAAGAGACTTTTGAGTATAGATACAAAAAGTGTTTTGGAGTTCCT GGAGATTTTAATCTTTCCCTCTTAGAGTACTTATATTCACCATCAGT TGAATCTGCAGGATTGAGATGGGTTGGTACTTGTAATGAACTAAATG CTGCTTATGCAGCAGATGGTTATAGCAGATATTCAAATAAAATAGGG TGTCTTATAACAACCTTATGGAGTAGGCGAGTTATCTGCATTGAATGG AATAGCAGGATCATTTGCAGAAAATGTTAAAGTGCTTCACATTGTAG GCGTTGCTAAGAGCATAGATAGCCGATCTTCAAATTTCTCAGATAGA AATCTACATCATCTTGTTCACAACCTACATGATTCAAACCTCAAAGG ACCTAATCATAAAGTTTATCATGATATGGTAAAAGATAGGGTAGCAT GCTCTGTTGCTTATCTTGAAGACATAGAACTGCTTGTGATCAAGTT GATAATGTAATAAGAGATATATACAAATACTCAAACCGGATATAT ATTTGTTCCAGCAGACTTTGCAGATATGTCAGTTACATGTGATAACT TAGTTAATGTACCAAGAATAAGTCAACAGGATTGCATAGTTTATCCA

		TCTGAAAATCAGTTAAGTGACATTATAAATAAAATAAAGTTCTCTGGAT TTACAGTTCTAAAACCCAGCCATTTTAGGTGATGTATTAAGTATA GATATGGTGTATCAAATTTTAAATAAACTTATATGTAAGACTGGA ATCTGGAATTTTCAACTGTTATGGGTAAATCAGTTATAGATGAAAG TAATCCAACCTTACATGGGTCAATATAATGGAAAAGAAGGTCTTAAAC AAGTGTATGAACATTTTGGAGCTTTGTGATTTAGTGCTTCACTTTGGT GTGGACATAAACGAAATTAACAATGGACATTATACTTTTACTTATAA ACCTAACGCAAAAATAATACAATTTTCACTCAAACTATATAAGACTTG TAGATACAAGACAAGGAAATGAGCAAATGTTCAAAGGCATAAACTTT GCTCCTATACTAAAAGAGCTGTATAAAAGAATAGATGTATCAAAGTT GTCACCTCAGTATGATTCAAATGTAACCTCAATACATAATGAGACAA TGAGATTAGAAGATCCCACTAATGGACAATCTTCAATTATTACCCAG GTACATCTTCAAAAACGATGCCAAAATTCCTCAATCCAGGAGATGT TGTGTCTGTGAAACAGGTTCTTTTCAATTTTCTGTGAGGGATTTTG CATTCCTTCTCAATTAATAATATATATCTCAAGGATTTTCTCTTCA ATCGGTATGGCATTACCAGCTGCATTGGGAGTTGGAATAGCAATGCA AGACCATTCAAATGCTCATATAAATGGCGGCAATGTTAAAGAGGATT ATAAACCAAGACTTATTTTATTTGAAGGAGATGGTGCCTGCACAAATG ACAATTCAAGAACTATCTACAATATTAATAATGTAATATTCCTCTTGA AGTAATAATTTGGAACAACAATGGATATACTATTGAAAGAGCAATAA TGGGTCCAACAGATCTTATAATGATGTAATGTCTGTGAAATGGACT AAATTATTTGAGGCATTTGGTGATTTTCGATGGAAAGTATACAAATTC TACTTTGATACAGTGTCTTCAAAGTTAGCTTTAAACTTGAAGAAC TCAAGAACTCAAATAAAAGATCTGGAATAGAATTATTAGAAGTTAAA CTTGGAGAGTTAGACTTCCCTGAGCAACTAAAATGCATGGTAGAAGC AGCAGCACTTAAGAGAAACAAAAATAA
5	LZ1561 codon adapted nucleotide sequence of phenylacetaldehyde reductase (<i>blPAR</i>) from <i>Brevibacterium linens</i>	ATGAAAGCAAGTTTGGCAACGGCAATTGGCGGCGAATTCACAGTACA TGACGTAGTAATAGATGACCCACAAGGAAGAGAGGTCTTGTGGATG TGAAAGCTTCAGGATTATGTCTTCTGATTTACACTTAATAGATCAT GATTTTCGGTCTCCCTCTTCCAGCTGTAGGCGGCCATGAAATTTTCAAG TGAGTGAGAAGTGTAGGACCAGGCGTTACCAGTATGTCTGTTGGAG ACCATGTTGTAGCTTGTCTTATAACTTTTGGCGGTGCATGTGCAGAA TGCTTTTCAAGGAAAACTACTTTATGTTCAAATCCTACTGCTGTAGC AAGAAAAGAAGGAGAAAAGCCAAGAGTTTCATTCCCTGATGGTCAGG AAATTGCACAATCAGTTAATGTTGGCGGCTTTCAGAACAAAGTACTA GTTTCATGAAATCAGCTTGCAGTAGTAACAATCAGATACCTTTCCC TCAGGCAGCACTTTTAGGGTGCTCAGTTGTACAGGAGCAGGAGCAG CTATAAATACAGCTCATGTAAGACCAGGGGATACAGTAGCGGTTATA GGAACAGGCGGCATAGGATTAAACGCAATAAGCGGAGCAAGATTAGC AGGGGCTAAGCACATTATAGCTATTGATATAGTTGATTCTAAATTAG AGGCTGCAAAAAGTTTGGAGCTACAGATCTTATAAATTCATCAACT ACTGATCCAGTGGCAGCAGTTCAAGAATTAAGTGGCGGCGTAGATCA TGCATTTGAAGTAATTGGATTAGAAGCTACGCAGAGGCAAGTTTCAAG AATTAACAAAACAGGCGGCACGGCATATTTAATAGGCATAGCACCA CCAGGAACAATACTGAATTTACATCATCATTAGATAGTTTGTTCG TCAAAGAAGACTGCAGGCTGTTTTGATGGGTAGTAGTAATGTTAAAA GAGATATAGCATTATATGCAGACTTGTATGTTTCAAGGAGCTTTTGAA TTAGATCATTTAGTATCAAGAGAAATATCCATAAATGAGATAAATGA TGGTTATGAAGCATTAATAAAGGTGAAGTTATACGTTTCAAGTTATAA CCAGTTTTTTAA
6	LZ1561 codon adapted nucleotide sequence of phenylacetaldehyde reductase (<i>ecPAR</i>) from <i>Escherichia coli</i>	ATGTCAATGATAAAATCATATGCAGCAAAAGAAGCAGGCGGCGAATT AGAAGTTTACGAATATGATCCAGGGGAATTAAGGCCTCAAGATGTAG AAGTTCAAGTTGATTACTGTGGAATATGCCATTCCGATTTATCTATG ATAGATAATGAATGGGATTTAGTCAGTATCCTCTTGTAGCAGGACA TGAAGTTATTGGCAGAGTTGTAGCTCTTGGTTTCAAGCAGCTCAGGATA AGGGCTTACAGGTAGGTGAGAGTAGGGATAGGCTGGACTGCCCGT TCGTGTGGACACTGCGATGCATGTATCAGTGGAATCAAATAAATTG TGAAACAAGGTGCAGTTCCAACATAATGAATAGGGCGGCTTTGCAG AAAAATTAAGAGCAGATTGGCAATGGTAATTCCTCTTCCAGAAAAC ATAGATATAGAATCAGCAGGTCCTCTCTTTGTGGCGGCATTACAGT

		ATTTAAACCTCTTCTTATGCATCATATTACTGCTACATCAAGAGTAG GAGTAATCGGTATAGGCGGCCTTGGACATATAGCAATTAACTTCTT CATGCTATGGGTGTGAAGTTACGGCATTAGTTCAAATCCAGCTAA AGAGCAAGAAGTTCTTGCTATGGGTGCCGACAAGGTAGTTAACAGTA GGGATCCACAGGCACTGAAAGCACTGGCGGGACAATTTGATCTTATA ATAAATACAGTAAACGTTTTCTCTAGATTGGCAACCATATTTTGAGGC ATTAACCTTATGGCGGCAATTTCCATACAGTTGGAGCCGTTCTTACAC CACTATCTGTTCCAGCTTTTACACTTATAGCAGGAGATCGTAGTGTT TCCGGCTCTGCAACTGGTACTCCTTATGAACTTAGAAAACCTTATGAG ATTTGCAGCAAGATCTAAAGTTGCACCTACCACAGAGCTTTTCCCTA TGAGCAAGATAAATGATGCAATTCAGCACGTTAGAGATGGAAGCA AGATATAGGGTTGTGTTAAAGGCTGATTATTAG
7	LZ1561 codon adapted nucleotide sequence of phenylacetylhydrolase (<i>rrPAR</i>) from <i>Rhodococcus ruber</i>	ATGAAAGCTTTGCAATATACAGAAATAGGAAGTGAACCAAGTAGTAGT AGATGTACCAACACCAGCGCCAGGACCAGGAGAGATCTTATTAAAGG TAAGTGCAGCAGGACTTTGTTCATTAGATATATTTGTAATGGATATG CCTGCTGAACAGTATATTTATGGCCTTCCATTAACGCTTGGCCATGA GGGTGTTGGAACAGTTGCAGAAATAGGGGCAGGTGTAACAGGATTTG AAACAGGTGATGCTGTTGCTGTTTATGGACCTTGGGGATGCGGTGCC TGCCATGCTTGTGCCCGTGAAGGGAAACTATTGTACTAGAGCTGC AGAGTTAGGAATAACACCCCTGGACTGGGATCACCTGGAAGTATGG CAGAGTATATGATTGTAGATTCTGCAAGACATTTAGTTCCATATAGGT GATTTGGATCCTGTTGCAGCTGTTCTCTTACAGATGCTGGATTGAC ACCTTATCATGCAATAAGTAGAGTGTTACCGCTTCTTGGACCAGGAT CTACAGCTGTAGTTATAGGAGTTGGCGGCTTAGGACATGTTGGCATC CAAATATTAAGAGCAGTGTGAGCAGCAAGAGTTATAGCAGTTGATTT AGATGATGACAGATTAGCTTTAGCAAGAGAAGTTGGTGCAGATGCAG CTGTTAAAAGTGGAGCAGGAGCAGCAGATGCTATTTCGTGAACCTACA GGCGGCGAAGGAGCAACTGCTGTGTTTGTATTTGTAGGAGCTCAGAG TACAATTGATACTGCACAGCAGGTAGTAGCAATAGACGCCATATAT CCGTAGTTGGTATTCATGCAGGTGCTCATGCAAAAGTAGGTTTTTTT ATGATACCTTTTGGAGCTTCTGTTGTAACCTTACTGGGGTACCCG TTCTGAACCTTATGGATGTAGTAGATCTTGCAAGAGCAGGCAGACTTG ATATACATACTGAGACTTTTACTTTAGATGAAGGTCCGACTGCATAT AGAAGACTAAGAGAAGGTTCCATAAGAGGTAGGGGAGTAGTAGTGCC TGGATAA
8	LZ1561 codon adapted nucleotide sequence of phenylacetylhydrolase (<i>rsPAR</i>) from <i>Rhodococcus</i> sp. ST-10	ATGAAAGCAATTCAATACACAAGAATAGGAGCAGAACCAGAACTTAC AGAAATACCAAAACCAGAGCCAGGACCAGGGGAAGTTCTTCTGAAG TAAGTGCAGCTGGAGTATGTACAGTGATGATTTTATAATGTGCGCTG CCTGAAGAACAGTATACATATGGACTACCACTTACATTAGGTCATGA AGGTGCAGGAAAAGTAGCAGCTGTAGGTGAGGGAGTAGAAGGTTTAG ACATAGGAACATATGTAGTAGTATATGGACCTTGGGGATGTGGAAT TGCTGGCACTGCTCTCAAGGCTTGGAAACTACTGTTCAAGAGCTCA GGAACCTTGAATAAATCCACCTGGACTTGGTGCACCAGGTGCATTAG CTGAATTTATGATTGTAGATTCACCACGTCATTTAGTGCCATAGGA GATTTGGATCCTGTTAAACTGTACCACTAAGTATGCAGGACTTAC ACCTTATCATGCTATAAAAAGAAGTTTACCAAAGTTAAGAGGCGGCT CCTATGCCGTTGTAATAGGAACAGGCGGCCTTGGACATGTAGCTATC CAATTATTAAGACATTTGTCTGCAGCTACTGTTATAGCACTTGACGT TTCAGCAGATAAATTGGAACCTGCTACTAAGGTAGGTGCACATGAAG TTGTATTATCAGACAAGGATGCAGCTGAAAATGTAAGGAAAATTACA GGATCACAAGGAGCAGCCTTAGTTTTAGATTTTGTGGATATCAACC TACAATTGACACTGCTATGGCTGTAGCTGGTGTGGAAAGTGATGTTA CAATAGTAGGTATAGGTGATGGTCAAGCTCATGCAAAGGTAGGTTTC TTTCAAAGTCTTATGAAGCTTCAGTAACTGTACCTTATTGGGGAGC TAGAAATGAGTTAATAGAACTCATAGATTAGCTCATGCAGGTATAT TTGATATTTCAAGTAGAACTTTTTTCACTTGACAATGGCGCAGAAGCA TATAGAAGATTAGCTGCTGGAACACTTTTCAAGGAAGAGCAGTAGTAGT TCCAGGATTATAA

9	LZ1561 codon adapted nucleotide sequence of phenylacetaldehyde reductase (<i>lePAR</i>) from <i>Solanum lycopersicum</i>	ATGTCAGTTACAGCAAAAACAGTTTGTGTTACAGGTGCTTCAGGTTA CATTGCTTCATGGTTAGTTAAATTTTTACTTCATTCTGGATATAATG TTAAGGCATCTGTTAGGGATCCAAATGATCCTAAAAAGACCCAGCAT CTATTAAGTCTTGGCGGCGCTAAAGAAAGGCTTCACCTATTTAAGGC AAATCTACTTGAAGAAGGTAGCTTTGATGCTGTTGTAGATGGTTGTG AAGGAGTATTCCACACTGCGTCACCATTTTATTACTCTGTAAGTAC CCACAAGCTGAATTGCTTGATCCAGCTGTAAAAGGTACACTGAACTT GTTAGGAAGTTGTGCAAAGGCACCAAGCGTAAAGAGAGTTGTATTAA CTAGCAGTATTGCTGCTGTTGCATACTCTGGTCAGCCAAGAACCCCA GAAGTAGTTGTAGATGAATCATGGTGGACATCACCAGATTACTGTAA AGAAAAACAACCTTTGGTATGTTTAAAGTAAAACCTTTAGCTGAAGAT CTGCTTGGAATTTGTTAAAGAAAAGGGAATAGATATGGTTGTAGTT AATCCAGCTATGGTTATTGGACCACTTTTGCAGCCAACATTAAATAC TAGCTCTGCAGCAGTACTTTCACTTGTAATGGTGTGAGACATACC CAAATTCAGTTTGGATGGGTAAATGTAAAGGACGTAGCAAATGCT CATATATTAGCATTTGAAAATCCATCCGCCAATGGAAGATATCTTAT GGTAGAAAGAGTAGCTCATTATTCTGATATATTGAAAATTTTAAGAG ATCTATATCCAACTATGCAGTTACCAGAAAAGTGTGCAGATGATAAT CCTCTTATGCAAAATTATCAGGTTTCAAAAGAAAAGGCCAAATCTTT AGGAATAGAATTTACGACTTTAGAAGAATCTATCAAAGAACTGTTG AATCACTAAAGGAGAAAAAATCTTTGGCGGCTCGTCTCCATGTAA
10	LZ1561 codon adapted nucleotide sequence of <i>AroG</i> from <i>Escherichia coli</i>	ATGAATTATCAAAATGATGATTTAAGAATAAAAGAAATTAAGAATT ATTACCTCCTGTAGCTTTATTAGAAAAATTTCTGCACTGAAAATG CAGCAAATACTGTAGCACATGCAAGAAAAAGCAATACATAAAATACTT AAAGGTAATGATGATAGATTATTAGTAGTAATAGGACCTTGTAGTAT ACATGATCCTGTAGCAGCAAAAGAATATGCAACTAGACTTTTAGCAT TAAGAGAAGAATTAAAAGATGAATTAGAATAAGTAATGAGAGTATAT TTTGAAAACCTAGAACTACTGTAGGATGGAAAGGACTTATAAATGA TCCTCATATGGATAATAGTTTCAAATAAATGATGGACTTAGAATAG CAAGAAAATTACTTTTAGATATAAATGATAGTGGATTACCTGCAGCT GGTGAATTTTGTAGATATGATAACTCCTCAATATTTAGCAGATTTAAT GAGTTGGGGAGCAATTGGAGCAAGAACTACTGAAAGTCAAGTACATA GAGAAGATGCAAGTGGACTTAGTTGTCCTGTAGGATTTAAAAATGGA ACTGATGGAACATAAAAGTAGCAATAGATGCAATAAATGCAGCTGG TGACCTCATTGTTTTCTTAGTGTAACAAAATGGGGACATAGTGCAA TAGTAAATACTAGTGGAAATGGTGATTGTATATAATACTTAGAGGT GGAAAAGAACCTAATTATTCTGCAAAACATGTAGCAGAAGTAAAAGA AGGACTTAATAAAGCTGGACTTCTGCACAGGTAATGATAGATTTT CTCATGCAATAGTAGTAACAATTTAAGAAACAAATGGATGTATGT GCAGATGTATGTAGCAAAATAGCTGGAGGTGAAAAGCAATAATTGG AGTAATGGTAGAAAGTCATTTAGTAGAAGGTAATCAAAGTTTAGAAA GTGGTGAACCTTTAGCTTATGGAAAAGTATAACTGATGCATGTATA GGATGGGAAGATACTGATGCACTTCTTAGACAACCTTGCAAATGCAGT AAAAGCAAGAAGAGGATAA
11	LZ1561 codon adapted nucleotide sequence of <i>ecAroE</i> from <i>Escherichia coli</i>	ATGGAACTTATGCAGTATTTGGCAATCCTATAGCACATTCAAAATC ACCATTTATACATCAACAATTTGCTCAGCAATTAATATTGAACATC CTTATGGAAGAGTTTTAGCTCCAATAAATGATTTTATAAATACTTTA AATGCTTTTTTTTCAGCAGGCGGCAAAGGAGCAATGTAAGTGTACC TTTTAAAGAGGAAGCTTTTGCCAGAGCAGATGAGTTAACTGAAAGAG CAGCATTAGCTGGTGCAGTTAATACATTGATGAGACTAGAAGATGGT AGGCTTTTAGGAGATAATACTGATGGGGTAGGTCTTTTGTCTGATCT TGAAAGACTTTCTTTTATAAGACCTGGCCTCCGGATCCTTTAATAG GTGCAGGCGGCGCATCCAGGGGAGTATTACTTCCATTACTATCACTG GATTGTGCAGTTACTATCACTAACAGAACAGTTTCAAGAGCTGAAGA GCTTGCTAAATTATTTGCACATACAGGATCAATCCAGGCACTTTCCA TGGATGAAC TAGAGGGACATGAATTTGACTTAATTATAAATGCGACT AGCAGTGGTATAAGTGGGGATATACCAGCTATTCCCAGCTCTTTAAT ACATCCTGGAATATACTGCTATGATATGTTTATCAAAAAGGTAAGA CACCCTTCTTAGCTTGGTGTGAACAAAGAGGAAGCAAGAGAAATGCA GATGGTCTTGGCATGCTGGTTGCACAAGCAGCTCATGCATTTTTACT

		ATGGCATGGAGTTTACCTGATGTTGAACCTGTTATAAAACAGCTGC AAGAAGAATTGTCAGCATAA
12	LZ1561 codon adapted nucleotide sequence of <i>cgAroE</i> from <i>Corynebacterium</i> <i>glutamicum</i>	ATGGGATCACATATAACTCACAGAGCAGCAGTTTTAGGCTCACCAAT TGAACATTCAAATCACCAGTGCTTCATAATACAGGATATAAAGCTT TAGGACTTGATCAATGGGAATATGACAGGTTTGAATGCACAGGAGAT ATGTTACCGGGCATAGTTTCAGGAGCAGATGAAACTTATCGAGGATT TTCTGTTACAATGCCTTCTAAATTTGCTGCTTTAGAATTTGCAGATG AAGTAAGTAAAGAGCAAGAGCTATTGGATCAGCAAATACATTACTT AGAACTGAAACAGGATGGAGAGCGGATAATACTGATGTGGATGGAAT AAGAGGTGCATTAGGTGAATTGTTAGGAAGTGCATCTCTTGCAGGAA AACATGCTATTGTAATAGGATCAGGCGGCACTGCAAGACCTGCAATT TGGGCACTTATAGAAGCAGGAGTAGCAAGAATAACTGTACTTAATAG ATCAGATAGAAGTCTGAACTTCAAACCTTATTTGATGAAACACCAA CTACGTTAGCATATGCTCCACTTGAGCACTTGGATATTGAAGCTGAC GTTGTAGTTTCTACTGTTCCCTTCAGCTGCTATAGCTGGTCTTGAAGA TACACTGGCTATAGCACCAGTGTGGATGTTATATATGATCCATGGC CAACTCCTTTAGTAGAAGTTGCAAGAGCTAAAGGACTTAAGGCTGTA GGCGGCCACGTAATGTTGGCCCATCAATCATATGGTCAATTTGAACA GTTTACAGGAATGGATGCTCCAAGGGATGCAATGAGAGAAGCTTTAG AAGAATCACTTGAATAAGTGAGGAACATTAG
13	LZ1561 codon adapted nucleotide sequence of <i>pheA1</i> from <i>Escherichia</i> <i>coli</i>	ATGACTAGTGAATCCATTACTTGCTTTAAGAGAAAAATATCTGC CCTTGATGAAAAATTACTTGCTTTATTAGCAGAAAGAGAGCTTG CAGTTGAGGTAGGTAAGGCAAAGTTGCTTTCGCACAGGCTGTTAGA GATATTGATAGGGAAGAGATCTTTTGGAAAGATTAATAACTCTTGG TAAGGCTCATCATTTGGATGCTCATTATATAACAAGATTATTTTCAGC TCATAATAGAAGACTCAGTTCCTACTCAACAGGCATTGCTTCAACAA CATTTGAATAAGATCAATCCTCACTCAGCCAGAATAGCTTTTTTAGG CCCCAAAGGTTCTATTACATCTGGCTGCTAGACAATATGCAGCAA GGCATTTTGAACAGTTTCATTGAATCAGGGTGTGCTAAATTTGCAGAT ATATTTAACCAGGTAGAAACAGGTCAAGCCGATTATGCTGTAGTACC TATAGAAAATACGTCGAGTGGTCTATTAATGATGTATATGATCTTT TACAGCACACTAGCCTATCCATAGTAGGAGAAATGACTTTAACTATA GATCACTGCCTTTTAGTATCAGGTACTACAGATCTTTCAACTATAAA TACAGTATATTCTCATCCACAGCCATTTCAACAATGTAGTAAATCC TAAACAGATATCCTCACTGGAAGATTGAATATACTGAAAGTACCAGT GCAGCAATGGAAAAAGTTGCACAGGCTAAATCACCACATGTAGCTGC ATTAGGTTCTGAGGCTGGCGGCACGTTGTATGGATTACAAGTACTAG AAAGAATAGAAGCCAATCAAAGGCAAAATTTTACAAGATTTGTGGTT TTGGCAAGAAAAGCAATAAATGTTTCAGATCAAGTCCCAGCAAAAAC TACATTACTAATGGCTACAGGACAACAGGCTGGGGCTTTAGTTGAGG CTTTATTAGTGTGAGAAATCACAATTTAATAATGACTAGGCTTGAA TCAAGACCTATTCATGGAATCCTTAA
14	LZ1561 codon adapted nucleotide sequence of <i>pheA2</i> from <i>Escherichia</i> <i>coli</i>	ATGACATCAGAAAATCCATTATTGGCACTTAGAGAAAAGATATCAGC ACTTGACGAAAAATTACTTGCAATTATTGGCAGAGAGAAGAGAGCTTG CTGTTGAAGTTGGAAAGGCTAAGCTGTTGTCCCACCGTCCAGTTAGA GATATTGACAGAGAACGGGACTTATTAGAAAGACTCATTACATTAGG AAAGGCTCATCATTTGGATGCATTATATTACAAGATTATTTTCAGC TCATAATAGAAGATTCACTATTAACCAACAGGCTTTACTTCAACAG CACTTAAATAAAATAAATCCACATTCACTAGGATTGCAATTTCTTAGG ACCAAAAGGAAGTTATAGTCATCTTGCTGCAAGACAATATGCAGCAA GACACTTTGAACAATTTATTGAATCAGGATGTGCTAAATTTGCAGAT ATCTTCAATCAAGTAGAAACAGGTCAAGGCTGATTATGCAGTAGTTCC AATAGAAAATACGTCAGCGGTGCTATAAATGATGTATATGATTTAC TTCAGCATACAAGCTTATCTATAGTTGGTGAGATGACATTGACTATA GATCACTGCTTACTTGTAAGTGGAACTACAGATTTGTCCACTATAAAA TACTGTTTACAGCCATCCACAGCCATTTCAACAGTGTAGTAAATTTT TAAATAGATATCCACACTGGAAAAATAGAATACACAGAAAGTACTTCC GCAGCAATGGAAAAGGTTGCACAGGCAAAGTCTCCTCAGTTGCAGC ATTAGGTAGTGAAGCAGGCGGCACACTTTATGGACTTCAAGTACTCG

		AAAGGATTGAAGCAAACCAAGGCAAAATTTTACTAGATTTGTTGTA CTTGCCAGAAAGGCTATAAATGTAAGTGATCAGGTACCAGCTAAAC TACTTTACTTATGGCAACAGGACAACAGGCAGGAGCACTAGTAGAAG CACTTTTAGTATTGAGAAATCATAATCTCATAATGACGAGGTTAGAG TCAAGACCAATTCATGGTAATCCAAGAGAGGAAATGTTTTACCTTGA TATCCAAGCAAATCTTGAATCAGCAGAAATGCAGAAAGCATTAAAGG AACTGGGTGAAATAACAAGATCAATGAAAGTGCTTGGATGTTATCCT TCTGAAATGTGGTACCTGTAGATCCAACCTAA
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[0148] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety

5 herein. The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement that that prior art forms part of the common general knowledge in the field of endeavor in any country.

[0149] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the methods and compositions of the disclosure (especially in the context of the

10 following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”) unless otherwise noted. The term “consisting essentially of” limits the scope of a composition, process, or method to the specified materials

15 or steps, or to those that do not materially affect the basic and novel characteristics of the composition, process, or method. The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the term “about” means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated.

20 **[0150]** Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, any concentration range, percentage range, ratio range, integer range, size range, or thickness range is to be understood to include the

25 value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0151] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all

examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the methods and compositions of the disclosure and does not pose a limitation on the scope of the disclosed methods and compositions unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element
5 as essential to the practice of the disclosed methods and compositions.

[0152] Preferred embodiments of the disclosure are described herein. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosed methods and
10 compositions to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosed methods and compositions unless otherwise indicated herein or otherwise clearly contradicted
15 by context.

CLAIMS

1. A microorganism capable of producing 2-phenylethanol, wherein the microorganism comprises:
 - 5 (a) a heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde; and
 - (b) a heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol.
2. The microorganism of claim 1, wherein:
 - 10 (a) the heterologous enzyme that converts phenylpyruvate to phenylacetaldehyde is decarboxylase; and
 - (b) the heterologous enzyme that converts phenylacetaldehyde to 2-phenylethanol is phenylacetaldehyde reductase.
- 15 3. The microorganism of claim 2, wherein the decarboxylase is a phenylpyruvate-specific decarboxylase.
4. The microorganism of claim 1, wherein the microorganism is a C1-fixing microorganism.
- 20 5. The microorganism of claim 1, wherein the microorganism is a Wood-Ljungdahl microorganism.
6. The microorganism of claim 1, wherein the microorganism is a bacterium.
- 25 7. The microorganism of claim 1, wherein the microorganism is a member of a genus selected from the group consisting of *Acetobacterium*, *Alkalibaculum*, *Blautia*, *Butyrivacterium*, *Clostridium*, *Eubacterium*, *Moorella*, *Oxobacter*, *Sporomusa*, and *Thermoanaerobacter*.
- 30 8. The microorganism of claim 1, wherein the microorganism is natively capable of producing phenylpyruvate.

9. The microorganism of claim 1, wherein the microorganism comprises a Wood-Ljungdahl pathway that converts CO, CO₂, and/or H₂ to acetyl-CoA.
10. The microorganism of claim 1, wherein the microorganism comprises one or more of:
- 5 (c) a native enzyme that converts acetyl-CoA to pyruvate;
 - (d) a native enzyme that converts pyruvate to phosphoenolpyruvate;
 - (e) a native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate;
 - (f) a native enzyme that converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquinate;
 - 10 (g) a native enzyme that converts 3-dehydroquinate to 3-dehydroshikimate;
 - (h) a native enzyme that converts 3-dehydroshikimate to shikimate;
 - (i) a native enzyme that converts shikimate to shikimate 3-phosphate;
 - (j) a native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate;
 - 15 (k) a native enzyme that converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate; or
 - (l) a native enzyme that converts chorismate to phenylpyruvate.
- 20 11. The microorganism of claim 10, wherein:
- (c) the native enzyme that converts acetyl-CoA to pyruvate is pyruvate:ferredoxin oxidoreductase;
 - (d) the native enzyme that converts pyruvate to phosphoenolpyruvate is pyruvate phosphate dikinase;
 - 25 (e) the native enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase;
 - (f) the native enzyme that converts 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate to 3-dehydroquinate is 3-dehydroquinase synthase;
 - 30 (g) the native enzyme that converts 3-dehydroquinase to 3-dehydroshikimate is 3-dehydroquinase dehydratase;
 - (h) the native enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase;

- (i) the native enzyme that converts shikimate to shikimate 3-phosphate is shikimate kinase;
- (j) the native enzyme that converts shikimate 3-phosphate to 5-O-(1-carboxyvinyl)-shikimate 3-phosphate is 5-enolpyruvylshikimate 3-phosphate synthase;
- (k) the native enzyme that converts 5-O-(1-carboxyvinyl)-shikimate 3-phosphate to chorismate is chorismate synthase; or
- (l) the native enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

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12. The microorganism of claim 1, wherein the microorganism further comprises one or more of:

- (e) a heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate;
- (h) a heterologous enzyme that converts 3-dehydroshikimate to shikimate; or
- (l) a heterologous enzyme that converts chorismate to phenylpyruvate.

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13. The microorganism of claim 12, wherein:

- (e) the heterologous enzyme that converts phosphoenolpyruvate and erythrose-4-phosphate to 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate is 2-dehydro-3-deoxy-D-arabino-heptonate 7-phosphate synthase;
- (h) the heterologous enzyme that converts 3-dehydroshikimate to shikimate is shikimate dehydrogenase; or
- (l) the heterologous enzyme that converts chorismate to phenylpyruvate is bi-functional chorismate mutase/prephenate dehydratase.

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14. The microorganism of any one of claims 1-13, wherein the microorganism comprises:

- (1) ecAroE, kivD, lePAR, pheA2, and aroG;
- (2) ecAroE, abPPDC, ecPAR, pheA1, and aroG;
- (3) cgAroE, abPPDC, lePAR, pheA2, and aroG;
- (4) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- (5) cgAroE, kivD, rrPAR, pheA2, and aroG;
- (6) ecAroE, abPPDC, lePAR, pheA2, and aroG;
- (7) pheA1, aroG, abPPDC, and ecPAR;

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- 5 (8) egAroE, abPPDC, lePAR, pheA1, and aroG;
 (9) ecAroE, abPPDC, ecPAR, pheA2, and aroG;
 (10) cgAroE, aro10, ecPAR, pheA2, and aroG;
 (11) cgAroE, abPPDC, lePAR, pheA1, and aroG;
 (12) cgAroE, aro10, lePAR, pheA2, and aroG;
 (13) ecAroE, abPPDC, lePAR, pheA1, and aroG;
 (14) ecAroE, aro10, ecPAR, pheA2, and aroG;
 (15) ecAroE, aro10, lePAR, pheA2, and aroG;
 (16) cgAroE, abPPDC, lePAR, pheA1, and aroG;
 10 (17) cgAroE, aro10, rsPAR, pheA2, and aroG;
 (18) cgAroE, abPPDC, blPAR, pheA2, and aroG;
 (19) ecAroE, kivD, blPAR, pheA2, and aroG;
 (20) cgAroE, aro10, blPAR, pheA2, and aroG;
 (21) ecAroE, abPPDC, blPAR, pheA2, and aroG;
 15 (22) ecAroE, abPPDC, adh6, pheA1, and aroG;
 (23) cgAroE, abPPDC, adh6, pheA2, and aroG;
 (24) cgAroE, abPPDC, ecPAR, pheA2, and aroG;
 (25) ecAroE, aro10, rsPAR, pheA2, and aroG;
 (26) ecAroE, aro10, adh6, pheA2, and aroG; or
 20 (27) ecAroE, abPPDC, ecPAR, pheA2, and aroG.

15. The microorganism of any one of claims 1-14, wherein the microorganism comprises the heterologous genes and promoters of combinatorial strain 2C03, 1C10, 2C54, 2C09, 2C47, 2C55, 2C53, H57, 1C47, 2C10, 2A18, 1C54, 2C01, 1C31, 2B17, 2C52, 1C09, H18,
 25 H58, 2C27, 2D22, 2D07, 2D24, 2D03, 1C29, 2B26, 2C38, 2A27, 2C12, or 2B27.

16. The microorganism of any one of claims 1-15, wherein the microorganism ferments a gaseous substrate comprising CO, CO₂, and/or H₂ to produce 2-phenylethanol.

30 17. The microorganism of claim 16, wherein the gaseous substrate comprises syngas or industrial waste gas.

18. The microorganism of claim 16, wherein the microorganism does not produce any other C₃+ alcohols.

19. The microorganism of claim 18, wherein the microorganism does not produce any other C3, C4, C5, C6, C7, C8, C9, or C10 alcohols.
20. A method of producing 2-phenylethanol comprising culturing the microorganism of
5 any one of claims 1-17 in the presence of a gaseous substrate.
21. The method of claim 20, wherein the gaseous substrate comprises a C1-carbon source comprising CO, CO₂, and/or H₂.
- 10 22. The method of any one of claims 20-22, wherein the gaseous substrate comprises syngas or industrial waste gas.