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(54) **Title**: CONTROLLED PRODUCTION OF CARBON-BASED PRODUCTS OF INTEREST

(57) **Abstract**: The present disclosure identifies methods and compositions for modifying photoautotrophic organisms as hosts, such that the organisms efficiently produce carbon- based products of interest, and in particular the use of such organisms for the commercial production of ethanol and related carbon-based molecules in the presence of contaminating microorganisms which can utilize the same. Other materials, methods, and compositions are also described.

CONTROLLED PRODUCTION OF CARBON-BASED PRODUCTS OF INTEREST

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 62/126,229, filed on February 27, 2015, which is herein incorporated by reference, in its entirety, for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 25, 2016, is named JBT-288.2PCT_SL.txt, and is 18,763 bytes in size.

BACKGROUND

[0003] Many existing photoautotrophic organisms (*i.e.*, plants, algae, and photosynthetic bacteria) are poorly suited for industrial bioprocessing and have therefore not demonstrated commercial viability. Recombinant photosynthetic microorganisms have been engineered to produce hydrocarbons and alcohols in amounts that exceed the levels produced naturally by the organism.

SUMMARY

[0004] Described herein is an engineered photosynthetic microorganism modified to increase internal storage of one or more nutrients relative to an otherwise identical control microorganism that is not modified to increase internal storage of the one or more nutrients, optionally wherein the one or more nutrients comprises phosphorus, sulfur, nitrogen, carbon, iron, manganese, cobalt, zinc, molybdenum, copper, boron, or combinations thereof, optionally wherein the engineered photosynthetic microorganism comprises one or more recombinant nucleotide sequences encoding one or more proteins capable of producing one or more carbon-based product(s) of interest, and optionally wherein the modification to increase internal storage of one or more nutrients is one or more modifications to the expression or regulation of *phoU* gene(s) or one or more modifications to the expression or regulation of phoU protein(s) of the microorganism, wherein the expression or activity of the protein encoded by the modified *phoU* gene(s) or the expression or activity of the phoU protein(s) can be or is reduced relative to an otherwise identical protein in an otherwise identical microorganism lacking the one or more modifications.

[0005] In some aspects, the expression or activity of the protein encoded by the *phoU* gene(s) is eliminated and/or knocked out.

[0006] In some aspects, the one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest comprise *adh*, *pdc*, *aar*, and/or *adm*. In some aspects, the one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest comprise *adh* and *pdc*. In some aspects, the one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest comprise *aar* and *adm*. In some aspects, the one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest are described in any of the following patent applications, each of which is herein incorporated by reference, in its entirety, for all purposes: PCT/US2008/075899 (filed 9/10/2008); PCT/US2008/083056 (filed 11/10/2008); PCT/US2009/035937 (filed 3/3/2009); PCT/US2009/055949 (filed 9/3/2009); PCT/US2010/039558 (filed 6/22/2010); PCT/US2010/041619 (filed 7/9/2010); U.S. Pat. No. 7,7949,69 (application filed 4/13/2010); PCT/US2011/051648 (filed 9/14/2011); U.S. Prov. App. No. 61/756,973 (filed 1/25/2013); U.S. Prov. App. No. 61/826,637 (filed 5/23/2013); U.S. Pat. Nos. 7,785,861, 7,794,969; 7,919,303; 7,955,820; 8,043,840; 8,101,397; 8,183,027; 8,481,285; 7,968,321; 8,048,666; 8,216,816; 7,981,647; 8,465,954; 8,048,654; 8,399,227; 8,227,237; and U.S. Ser. No. 13/370,654.

[0007] In some aspects, said one or more recombinant nucleotide sequences (e.g., genes or mRNA) are integrated into the genome of said microorganism. In some aspects, said one or more recombinant nucleotide sequences are extrachromosomal.

[0008] In some aspects, an inducible or repressible promoter operably linked to the coding sequence of the *phoU* gene(s). In some aspects, the expression profile of the *phoU* gene(s) can be altered via the promoter relative to the otherwise identical microorganism. In some aspects, the expression profile of the *phoU* gene(s) can be temporally altered via the promoter relative to the otherwise identical microorganism.

[0009] In some aspects, the expression profile of the *phoU* gene(s) can be altered via one or more modifications.

[0010] In some aspects, the one or more modifications comprise one or more mutations in the modified *phoU* gene(s). In some aspects, the modified *phoU* gene is a deleted *phoU* gene(s). In some aspects, the one or modifications comprise cleavage or silencing of the mRNA product(s) of the *phoU* gene(s) via RNA interference (RNAi). In some aspects, the

amount of mRNA product(s) of the phoU gene(s) is reduced. In some aspects, the microorganism further comprises one or more recombinant nucleotide sequences encoding a catalase or wherein the microorganism further comprises a catalase.

[0011] In some aspects, the carbon-based product(s) of interest comprises or is ethanol or an alkane. Other carbon-based product(s) of interest are decribed herein. Other carbon-based product(s) of interest are decribed in PCT/US2008/075899 (filed 9/10/2008); PCT/US2008/083056 (filed 11/10/2008); PCT/US2009/035937 (filed 3/3/2009); PCT/US2009/055949 (filed 9/3/2009); PCT/US2010/039558 (filed 6/22/2010); PCT/US2010/041619 (filed 7/9/2010); U.S. Pat. No. 7,7949,69 (application filed 4/13/2010); PCT/US2011/051648 (filed 9/14/2011); U.S. Prov. App. No. 61/756,973 (filed 1/25/2013); U.S. Prov. App. No. 61/826,637 (filed 5/23/2013); U.S. Pat. Nos. 7,785,861, 7,794,969; 7,919,303; 7,955,820; 8,043,840; 8,101,397; 8,183,027; 8,481,285; 7,968,321; 8,048,666; 8,216,816; 7,981,647; 8,465,954; 8,048,654; 8,399,227; 8,227,237; and U.S. Ser. No. 13/370,654.

[0012] In some aspects, the phosphate uptake rate of the microorganism is about 2, 3, 4, 5, 6, 7, 8, or 9 times the rate of the phosphate uptake in an otherwise identical microorganism cultured under identical conditions. In some aspects, the phosphate uptake rate of the microorganism is about 9 times the rate of the phosphate uptake in an otherwise identical microorganism cultured under identical conditions. In some aspects, the phosphate uptake rate of the microorganism is greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300 $\mu M\ h^{-1}$. In some aspects, the phosphate uptake rate of the microorganism is about 80 to about 300 $\mu M\ h^{-1}$.

[0013] In some aspects, the microorganism is capable of producing an equivalent rate and/or amount of carbon-based product(s) of interest in both the presence and absence of a distinct microorganism. In some aspects, the distinct microorganism is a carbon-based product(s) of interest catabolizing microorganism. In some aspects, the distinct microorganism comprises *Acinetobacter baylyi* ADP1, *Bacillus thuringiensis*, and/or *Pseudomonas stutzeri*. In some aspects, the distinct microorganism is present at 10 or greater than 10 colony forming units (CFU)/ml. In some aspects, the distinct microorganism is present at 10^3 CFU/ml.

[0014] In some aspects, the microorganism is capable of producing carbon-based product(s) of interest over an increased period of time in the presence of acetate. In some

aspects, the microorganism is capable of producing carbon-based product(s) of interest over an increased period of time in the presence of about 10 mmol acetate.

[0015] In some aspects, said microorganism is a cyanobacterium. In some aspects, said microorganism is a thermophillic or thermotolerant cyanobacterium. In some aspects, said microorganism is a *Synechococcus* species.

[0016] Also described herein is a cell culture comprising a culture medium and a microorganism disclosed herein. In some aspects, the culture further comprises acetate. In some aspects, the culture further comrpsies one or more distinct microorganisms. In some aspects, the one or more distinct microorganisms are carbon-based product(s) of interest catabolizing microorganisms. In some aspects, the distinct microorganism is present at 10 or greater than 10 CFU/ml. In some aspects, the distinct microorganism is present at 10^3 CFU/ml.

[0017] Also described herein is a method for producing carbon-based product(s) of interest, comprising: culturing an engineered photsynthetic microorganism described herein in a culture medium, wherein said engineered microorganism produces equivalent or increased amounts of carbon-based product(s) of interest relative to an otherwise identical microorganism, cultured under identical conditions. In some aspects, the method further comprises allowing the carbon-based product(s) of interest to accumulate in the culture. In some aspects, the method further comprises isolating at least a portion of the carbon-based product(s) of interest from said culture. In some aspects, the method further comprises processing the isolated carbon-based product(s) of interest to produce a processed compound.

[0018] Also described herein is a method for producing carbon-based product(s) of interest, comprising: (i) culturing an engineered photosynthetic microorganism disclosed herein in a culture medium; and (ii) exposing said engineered photosynthetic microorganism to light and inorganic carbon, wherein said exposure results in the conversion of said inorganic carbon by said microorganism into carbon-based product(s) of interest in an amount greater than or equal to that converted by an otherwise identical photosynthetic microorganism, cultured under identical conditions. In some aspects, the method further comprises allowing the carbon-based product(s) of interest to accumulate in the culture. In some aspects, the method further comprises isolating at least a portion of the carbon-based product(s) of interest from said culture. In some aspects, the method further comprises processing the isolated carbon-based product(s) of interest to produce a processed compound.

[0019] Also described herein is a composition comprising carbon-based product(s) of interest, wherein said carbon-based product(s) of interest are produced by a method described

herein. In some aspects, the composition comprises at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% carbon-based product(s) of interest.

BRIEF DESCRIPTION OF THE FIGURES

[0020] Figure 1. Growth of JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea.

[0021] Figure 2. Phosphate uptake by JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea.

[0022] Figure 3. Growth of JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea. The black arrow indicates the time of addition of 50 μM cumate to the induced flasks.

[0023] Figure 4. Phosphate consumption by JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea. The black arrow indicates the time of addition of 50 μM cumate to the induced flasks.

[0024] Figure 5. Ethanol production by JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea. The black arrow indicates the time of addition of 50 μM cumate to the induced flasks.

[0025] Figure 6. Growth of JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea. The black arrow indicates the time of addition of 20 μM cumate to all the cultures. Those flasks which received contaminants are indicated with the starting contaminant inoculation concentration in CFU/ml.

[0026] Figure 7. Ethanol production of JCC5048 and JCC5473 in phosphate containing JB3.0 medium containing urea. The black arrow indicates the time of addition of 20 μM cumate to all the cultures. Those flasks which received contaminants are indicated with the starting contaminant inoculation concentration in CFU/ml.

[0027] Figure 8. Ethanol production of JCC5473 in JB3.0 medium urea (base medium). The black arrow indicates the time of addition of 20 μM cumate to both cultures and 10 mM acetate (final concentration) to one flask.

[0028] Figure 9. Growth of JCC5473 in JB3.0 medium containing urea (base medium). The black arrow indicates the time of addition of 20 μM cumate to both cultures and 10 mM acetate (final concentration) to one flask.

[0029] Figure 10. Concentration of phosphate in the medium detected over time following inoculation of JCC5473 in JB3.0 media with either 1x, 2x, 3x, or 4x the standard phosphate level of JB3.0.

[0030] Figure 11. Growth of JCC5473 in JB3.0 base medium containing either 1x, 2x, 3x, or 4x the phosphate concentration of JB3.0.

[0031] Figure 12. Growth of JCC5473 in JB3.0 lacking phosphate. Prior to inoculation, cultures were grown in media containing either 1x, 2x, 3x, or 4x the phosphate of JB3.0 (source indicated in legend). The flask cultured in 4x phosphate was washed with JB3.0 lacking phosphate to ensure no phosphate carryover.

[0032] Figure 13. Ethanol productivity of JCC5473 in JB3.0 lacking phosphate. Prior to inoculation, cultures were grown in media containing either 1x, 2x, 3x, or 4x the phosphate of JB3.0 (source indicated in legend). The flask cultured in 4x phosphate was washed with JB3.0 lacking phosphate to ensure no phosphate carryover. The black arrow indicates the time of addition of 20 μM cumate.

[0033] Figure 14. Growth of JCC5473 in JB3.0 lacking phosphate. Prior to inoculation, cultures were grown in media containing either 1x, 2x, 3x, or 4x the phosphate of JB3.0 (source indicated in legend). The flask cultured in 4x phosphate was washed with JB3.0 lacking phosphate to ensure no phosphate carryover. The black arrow indicates the time of addition of 20 μM cumate.

[0034] Figure 15. Ethanol production of the cultures in JB3.0 medium containing urea. The black arrow indicates the time of addition of 20 μM cumate to both cultures and 10 mM acetate (final concentration) to one flask as indicated in the legend by NaAc. Both cultures of JCC5473 died before the end of the experiment and the final timepoint for the flasks indicates when they had turned white and were removed from the incubator.

[0035] Figure 16. Growth of the cultures in JB3.0 medium containing urea. The black arrow indicates the time of addition of 20 μM cumate to both cultures and 10 mM acetate (final concentration) to one flask as indicated in the legend by NaAc. Both cultures of JCC5473 died before the end of the experiment and the final timepoint for the flasks indicates when they had turned white and were removed from the incubator.

[0036] Figure 17. Phosphate consumption by JCC5290 and JCC5476 in JB3.0 medium containing urea over the first 5 days of the experiment. The black arrow indicates the time of addition of 20 μM cumate to the flasks.

[0037] Figure 18. Growth of JCC5048 and JCC5473 in JB3.0 media containing the indicated phosphate and 10mM acetate in an outdoor photobioreactor. The black arrow indicates the time of addition of 5 μM cumate.

[0038] Figure 19. Ethanol productivities of JCC5048 and JCC5473 in JB3.0 media containing the indicated phosphate and 10mM acetate in an outdoor photobioreactor. The black arrow indicates the time of addition of 5 μM cumate.

[0039] Figure 20. Growth of JCC5048 and JCC6082 in the FMT photobioreactor under diurnal light conditions of 200-2000 uE/m2/s. In the indicated culture JCC6082 was induced with 100 μM DOC following phosphate depletion. The black arrow indicates the time of addition of 5 μM cumate.

[0040] Figure 21. Ethanol productivity of JCC5048 and JCC6082 in the FMT photobioreactor under diurnal light conditions. In the indicated culture, JCC6082 was induced with 100 μM DOC following phosphate depletion. The black arrow indicates the time of addition of 5 μM cumate.

[0041] Figure 22. Daily ethanol productivity of JCC5048 and JCC6082 in the FMT photobioreactor under diurnal light conditions of 200-2000 uE/m2/s. In the indicated culture JCC6082 was induced with 100 μM DOC following phosphate depletion. The black arrow indicates the time of addition of 5 μM cumate.

DETAILED DESCRIPTION

[0042] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

[0043] The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Taylor and Drickamer, *Introduction to Glycobiology*, Oxford Univ. Press (2003); *Worthington Enzyme Manual*, Worthington Biochemical Corp., Freehold, N.J.; *Handbook of Biochemistry: Section A Proteins*, Vol I, CRC Press (1976); Handbook of Biochemistry: Section A Proteins, Vol II, CRC Press (1976); *Essentials of Glycobiology*, Cold Spring Harbor Laboratory Press (1999).

[0044] All publications, patents and other references mentioned herein are hereby incorporated by reference in their entireties.

[0045] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0046] The term "polynucleotide" or "nucleic acid molecule" or "nucleotide sequence" refers to a polymeric form of nucleotides of at least 10 bases in length. The term includes DNA molecules (*e.g.*, cDNA or genomic or synthetic DNA) and RNA molecules (*e.g.*, mRNA or synthetic RNA), as well as analogs of DNA or RNA containing non-natural nucleotide analogs, non-native intemucleoside bonds, or both. The nucleic acid can be in any topological conformation. For instance, the nucleic acid can be single-stranded, double-stranded, triple-stranded, quadruplexed, partially double-stranded, branched, hairpinned, circular, or in a padlocked conformation.

[0047] Unless otherwise indicated, and as an example for all sequences described herein under the general format "SEQ ID NO:", "nucleic acid comprising SEQ ID NO:1" refers to a nucleic acid, at least a portion of which has either (i) the sequence of SEQ ID NO:1, or (ii) a sequence complementary to SEQ ID NO:1. The choice between the two is dictated by the context. For instance, if the nucleic acid is used as a probe, the choice between the two is dictated by the requirement that the probe be complementary to the desired target.

[0048] An "isolated" RNA, DNA or a mixed polymer is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its natural host cell, *e.g.*, ribosomes, polymerases and genomic sequences with which it is naturally associated.

[0049] As used herein, an "isolated" organic molecule (e.g., an alkane or ethanol) is one which is substantially separated from the cellular components (membrane lipids, chromosomes, proteins) of the host cell from which it originated, or from the medium in which the host cell was cultured. The term does not require that the biomolecule has been separated from all other chemicals, although certain isolated biomolecules may be purified to near homogeneity.

[0050] The term "recombinant" refers to a biomolecule, *e.g.*, a gene or protein, that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a portion of a polynucleotide in which the gene is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature. The term "recombinant" can be used in reference to cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems, as well as proteins and/or mRNAs encoded by such nucleic acids.

[0051] As used herein, an endogenous nucleic acid sequence in the genome of an organism (or the encoded protein product of that sequence) is deemed "recombinant" herein if a heterologous sequence is placed adjacent to the endogenous nucleic acid sequence, such that the expression of this endogenous nucleic acid sequence is altered. In this context, a heterologous sequence is a sequence that is not naturally adjacent to the endogenous nucleic acid sequence, whether or not the heterologous sequence is itself endogenous (originating from the same host cell or progeny thereof) or exogenous (originating from a different host cell or progeny thereof). By way of example, a promoter sequence can be substituted (*e.g.*, by homologous recombination) for the native promoter of a gene in the genome of a host cell, such that this gene has an altered expression pattern. This gene would now become "recombinant" because it is separated from at least some of the sequences that naturally flank it.

[0052] A nucleic acid is also considered "recombinant" if it contains any modifications that do not naturally occur to the corresponding nucleic acid in a genome. For instance, an endogenous coding sequence is considered "recombinant" if it contains an insertion, deletion or a point mutation introduced artificially, *e.g.*, by human intervention. A "recombinant nucleic acid" also includes a nucleic acid integrated into a host cell chromosome at a heterologous site and a nucleic acid construct present as an episome.

[0053] As used herein, the phrase "degenerate variant" of a reference nucleic acid sequence encompasses nucleic acid sequences that can be translated, according to the standard genetic code, to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence. The term "degenerate oligonucleotide" or "degenerate primer" is used to signify an oligonucleotide capable of hybridizing with target nucleic acid sequences that are not necessarily identical in sequence but that are homologous to one another within one or more particular segments.

[0054] The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for

maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wis. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. Pearson, Methods Enzymol. 183:63-98 (1990) (hereby incorporated by reference in its entirety). For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference. Alternatively, sequences can be compared using the computer program, BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish and States, *Nature Genet.* 3:266-272 (1993); Madden *et al.*, *Meth. Enzymol.* 266:131-141 (1996); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang and Madden, *Genome Res.* 7:649-656 (1997)), especially blastp or tblastn (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)).

[0055] The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 76%, 80%, 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

[0056] Alternatively, substantial homology or similarity exists when a nucleic acid or fragment thereof hybridizes to another nucleic acid, to a strand of another nucleic acid, or to the complementary strand thereof, under stringent hybridization conditions. "Stringent hybridization conditions" and "stringent wash conditions" in the context of nucleic acid hybridization experiments depend upon a number of different physical parameters. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, solvents, the base composition of the hybridizing species, length of the complementary regions, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. One having ordinary skill in

the art knows how to vary these parameters to achieve a particular stringency of hybridization.

[0057] In general, "stringent hybridization" is performed at about 25°C below the thermal melting point (T_m) for the specific DNA hybrid under a particular set of conditions. "Stringent washing" is performed at temperatures about 5°C lower than the T_m for the specific DNA hybrid under a particular set of conditions. The T_m is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. See Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), page 9.51, hereby incorporated by reference. For purposes herein, "stringent conditions" are defined for solution phase hybridization as aqueous hybridization (*i.e.*, free of formamide) in 6xSSC (where 20xSSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% SDS at 65°C for 8-12 hours, followed by two washes in 0.2xSSC, 0.1% SDS at 65°C for 20 minutes. It will be appreciated by the skilled worker that hybridization at 65°C will occur at different rates depending on a number of factors including the length and percent identity of the sequences which are hybridizing.

[0058] The nucleic acids (also referred to as polynucleotides) of this present invention may include both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. They may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, intemucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, *etc.*), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), pendent moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen, *etc.*), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids, *etc.*) Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. Other modifications can include, for example, analogs in which the ribose ring contains a bridging moiety or other structure such as the modifications found in "locked" nucleic acids.

[0059] The term "mutated" when applied to nucleic acid sequences means that nucleotides in a nucleic acid sequence may be inserted, deleted or changed compared to a reference

nucleic acid sequence. A single alteration may be made at a locus (a point mutation) or multiple nucleotides may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid sequence. A nucleic acid sequence may be mutated by any method known in the art including but not limited to mutagenesis techniques such as "error-prone PCR" (a process for performing PCR under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product; see, *e.g.*, Leung *et al.*, Technique, 1:11-15 (1989) and Caldwell and Joyce, PCR Methods Applic. 2:28-33 (1992)); and "oligonucleotide-directed mutagenesis" (a process which enables the generation of site-specific mutations in any cloned DNA segment of interest; see, *e.g.*, Reidhaar-Olson and Sauer, Science 241:53-57 (1988)).

[0060] The term "attenuate" as used herein generally refers to a functional deletion, including a mutation, partial or complete deletion, insertion, or other variation made to a gene sequence or a sequence controlling the transcription of a gene sequence, which reduces or inhibits production of the gene product, or renders the gene product non-functional. In some instances a functional deletion is described as a knockout mutation. Attenuation also includes amino acid sequence changes by altering the nucleic acid sequence, placing the gene under the control of a less active promoter, down-regulation, expressing interfering RNA, ribozymes or antisense sequences that target the gene of interest, or through any other technique known in the art. In one example, the sensitivity of a particular enzyme to feedback inhibition or inhibition caused by a composition that is not a product or a reactant (non-pathway specific feedback) is lessened such that the enzyme activity is not impacted by the presence of a compound. In other instances, an enzyme that has been altered to be less active can be referred to as attenuated.

[0061] **Deletion**: The removal of one or more nucleotides from a nucleic acid molecule or one or more amino acids from a protein, the regions on either side being joined together.

[0062] **Knock-out:** A gene whose level of expression or activity has been reduced to zero. In some examples, a gene is knocked-out via deletion of some or all of its coding sequence. In other examples, a gene is knocked-out via introduction of one or more nucleotides into its open reading frame, which results in translation of a non-sense or otherwise non-functional protein product.

[0063] The term "vector" as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which generally refers to a circular double stranded DNA loop into which

additional DNA segments may be ligated, but also includes linear double-stranded molecules such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme. Other vectors include cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC). Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome (discussed in more detail below). Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain preferred vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply "expression vectors").

[0064] "Operatively linked" or "operably linked" expression control sequences refers to a linkage in which the expression control sequence is contiguous with the gene of interest to control the gene of interest, as well as expression control sequences that act in trans or at a distance to control the gene of interest.

[0065] The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to affect the expression of coding sequences to which they are operatively linked. Expression control sequences are sequences which control the transcription, post-transcriptional events and translation of nucleic acid sequences. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*e.g.*, ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0066] The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding

generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. A recombinant host cell may be an isolated cell or cell line grown in culture or may be a cell which resides in a living tissue or organism.

[0067] The term "peptide" as used herein refers to a short polypeptide, *e.g.*, one that is typically less than about 50 amino acids long and more typically less than about 30 amino acids long. The term as used herein encompasses analogs and mimetics that mimic structural and thus biological function.

[0068] The term "polypeptide" encompasses both naturally-occurring and non-naturally-occurring proteins, and fragments, mutants, derivatives and analogs thereof. A polypeptide may be monomeric or polymeric. Further, a polypeptide may comprise a number of different domains each of which has one or more distinct activities.

[0069] The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) exists in a purity not found in nature, where purity can be adjudged with respect to the presence of other cellular material (*e.g.*, is free of other proteins from the same species) (3) is expressed by a cell from a different species, or (4) does not occur in nature (*e.g.*, it is a fragment of a polypeptide found in nature or it includes amino acid analogs or derivatives not found in nature or linkages other than standard peptide bonds). Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A polypeptide or protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art. As thus defined, "isolated" does not necessarily require that the protein, polypeptide, peptide or oligopeptide so described has been physically removed from its native environment.

[0070] The term "polypeptide fragment" as used herein refers to a polypeptide that has a deletion, *e.g.*, an amino-terminal and/or carboxy-terminal deletion compared to a full-length polypeptide. In a preferred embodiment, the polypeptide fragment is a contiguous sequence in which the amino acid sequence of the fragment is identical to the corresponding positions in the naturally-occurring sequence. Fragments typically are at least 5, 6, 7, 8, 9 or 10 amino acids long, preferably at least 12, 14, 16 or 18 amino acids long, more preferably at least 20 amino acids long, more preferably at least 25, 30, 35, 40 or 45, amino acids, even more

preferably at least 50 or 60 amino acids long, and even more preferably at least 70 amino acids long.

[0071] A "modified derivative" refers to polypeptides or fragments thereof that are substantially homologous in primary structural sequence but which include, *e.g.*, in vivo or in vitro chemical and biochemical modifications or which incorporate amino acids that are not found in the native polypeptide. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, *e.g.*, with radionuclides, and various enzymatic modifications, as will be readily appreciated by those skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{125}I, ^{32}P, ^{35}S, and ^{3}H, ligands which bind to labeled antiligands (*e.g.*, antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well known in the art. See, *e.g.*, Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992, and Supplements to 2002) (hereby incorporated by reference).

[0072] The term "fusion protein" refers to a polypeptide comprising a polypeptide or fragment coupled to heterologous amino acid sequences. Fusion proteins are useful because they can be constructed to contain two or more desired functional elements from two or more different proteins. A fusion protein comprises at least 10 contiguous amino acids from a polypeptide of interest, more preferably at least 20 or 30 amino acids, even more preferably at least 40, 50 or 60 amino acids, yet more preferably at least 75, 100 or 125 amino acids. Fusions that include the entirety of the proteins of the present invention have particular utility. The heterologous polypeptide included within the fusion protein of the present invention is at least 6 amino acids in length, often at least 8 amino acids in length, and usefully at least 15, 20, and 25 amino acids in length. Fusions that include larger polypeptides, such as an IgG Fc region, and even entire proteins, such as the green fluorescent protein ("GFP") chromophore-containing proteins, have particular utility. Fusion proteins can be produced recombinantly by constructing a nucleic acid sequence which encodes the polypeptide or a fragment thereof in frame with a nucleic acid sequence encoding a different protein or peptide and then expressing the fusion protein. Alternatively, a fusion protein can be produced chemically by crosslinking the polypeptide or a fragment thereof to another protein.

[0073] The term "non-peptide analog" refers to a compound with properties that are analogous to those of a reference polypeptide. A non-peptide compound may also be termed a "peptide mimetic" or a "peptidomimetic." See, *e.g.*, Jones, *Amino Acid and Peptide Synthesis*, Oxford University Press (1992); Jung, *Combinatorial Peptide and Nonpeptide Libraries: A Handbook*, John Wiley (1997); Bodanszky *et al.*, *Peptide Chemistry--A Practical Textbook*, Springer Verlag (1993); *Synthetic Peptides: A Users Guide*, (Grant, ed., W. H. Freeman and Co., 1992); Evans *et al.*, *J. Med. Chem.* 30:1229 (1987); Fauchere, J. *Adv. Drug Res.* 15:29 (1986); Veber and Freidinger, *Trends Neurosci.*, 8:392-396 (1985); and references sited in each of the above, which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to useful peptides of the present invention may be used to produce an equivalent effect and are therefore envisioned to be part of the present invention.

[0074] A "polypeptide mutant" or "mutein" refers to a polypeptide whose sequence contains an insertion, duplication, deletion, rearrangement or substitution of one or more amino acids compared to the amino acid sequence of a native or wild-type protein. A mutein may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the naturally-occurring protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. A mutein may have the same but preferably has a different biological activity compared to the naturally-occurring protein.

[0075] A mutein has at least 85% overall sequence homology to its wild-type counterpart. Even more preferred are muteins having at least 90% overall sequence homology to the wild-type protein.

[0076] In an even more preferred embodiment, a mutein exhibits at least 95% sequence identity, even more preferably 98%, even more preferably 99% and even more preferably 99.9% overall sequence identity.

[0077] Sequence homology may be measured by any common sequence analysis algorithm, such as Gap or Bestfit.

[0078] Amino acid substitutions can include those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinity or enzymatic activity, and (5) confer or modify other physicochemical or functional properties of such analogs.

[0079] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology-A Synthesis* (Golub and Gren eds., Sinauer Associates, Sunderland, Mass., 2nd ed. 1991), which is incorporated herein by reference. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand end corresponds to the amino terminal end and the right-hand end corresponds to the carboxy-terminal end, in accordance with standard usage and convention.

[0080] A protein has "homology" or is "homologous" to a second protein if the nucleic acid sequence that encodes the protein has a similar sequence to the nucleic acid sequence that encodes the second protein. Alternatively, a protein has homology to a second protein if the two proteins have "similar" amino acid sequences. (Thus, the term "homologous proteins" is defined to mean that the two proteins have similar amino acid sequences.) As used herein, homology between two regions of amino acid sequence (especially with respect to predicted structural similarities) is interpreted as implying similarity in function.

[0081] When "homologous" is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of homology may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson, 1994, *Methods Mol. Biol.* 24:307-31 and 25:365-89 (herein incorporated by reference).

[0082] The following six groups each contain amino acids that are conservative substitutions for one another: 1) Serine (S), Threonine (T); 2) Aspartic Acid (D), Glutamic Acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I),

Leucine (L), Methionine (M), Alanine (A), Valine (V), and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[0083] Sequence homology for polypeptides, which is also referred to as percent sequence identity, is typically measured using sequence analysis software. See, *e.g.*, the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wis. 53705. Protein analysis software matches similar sequences using a measure of homology assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild-type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1.

[0084] A preferred algorithm when comparing a particular polypeptide sequence to a database containing a large number of sequences from different organisms is the computer program BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish and States, *Nature Genet.* 3:266-272 (1993); Madden *et al.*, *Meth. Enzymol.* 266:131-141 (1996); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang and Madden, *Genome Res.* 7:649-656 (1997)), especially blastp or tblastn (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)).

[0085] Preferred parameters for BLASTp are: Expectation value: 10 (default); Filter: seg (default); Cost to open a gap: 11 (default); Cost to extend a gap: 1 (default); Max. alignments: 100 (default); Word size: 11 (default); No. of descriptions: 100 (default); Penalty Matrix: BLOWSUM62.

[0086] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences. Database searching using amino acid sequences can be measured by algorithms other than blastp known in the art. For instance, polypeptide sequences can be compared using FASTA, a program in GCG Version 6.1. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. Pearson, *Methods Enzymol.* 183:63-98 (1990) (incorporated by reference herein). For example, percent sequence identity between

amino acid sequences can be determined using FASTA with its default parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

[0087] "Specific binding" refers to the ability of two molecules to bind to each other in preference to binding to other molecules in the environment. Typically, "specific binding" discriminates over adventitious binding in a reaction by at least two-fold, more typically by at least 10-fold, often at least 100-fold. Typically, the affinity or avidity of a specific binding reaction, as quantified by a dissociation constant, is about 10^{-7} M or stronger (*e.g.*, about 10^{-8} M, 10^{-9} M or even stronger).

[0088] The term "region" as used herein refers to a physically contiguous portion of the primary structure of a biomolecule. In the case of proteins, a region is defined by a contiguous portion of the amino acid sequence of that protein.

[0089] The term "domain" as used herein refers to a structure of a biomolecule that contributes to a known or suspected function of the biomolecule. Domains may be co-extensive with regions or portions thereof; domains may also include distinct, non-contiguous regions of a biomolecule. Examples of protein domains include, but are not limited to, an Ig domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

[0090] As used herein, the term "molecule" means any compound, including, but not limited to, a small molecule, peptide, protein, sugar, nucleotide, nucleic acid, lipid, *etc.*, and such a compound can be natural or synthetic.

[0091] "Carbon-based Products of Interest" include alcohols such as ethanol, propanol, isopropanol, butanol, fatty alcohols, fatty acid esters, wax esters; hydrocarbons and alkanes such as propane, octane, diesel, Jet Propellant 8 (JP8); polymers such as terephthalate, 1,3-propanediol, 1,4-butanediol, polyols, Polyhydroxyalkanoates (PHA), poly-beta-hydroxybutyrate (PHB), acrylate, adipic acid, ε-caprolactone, isoprene, caprolactam, rubber; commodity chemicals such as lactate, Docosahexaenoic acid (DHA), 3-hydroxypropionate, γ-valerolactone, lysine, serine, aspartate, aspartic acid, sorbitol, ascorbate, ascorbic acid, isopentenol, lanosterol, omega-3 DHA, lycopene, itaconate, 1,3-butadiene, ethylene, propylene, succinate, citrate, citric acid, glutamate, malate, 3-hydroxypropionic acid (HPA), lactic acid, THF, gamma butyrolactone, pyrrolidones, hydroxybutyrate, glutamic acid, levulinic acid, acrylic acid, malonic acid; specialty chemicals such as carotenoids, isoprenoids, itaconic acid; pharmaceuticals and pharmaceutical intermediates such as 7-aminodeacetoxycephalosporanic acid (7-ADCA)/cephalosporin, erythromycin, polyketides, statins, paclitaxel, docetaxel, terpenes, peptides, steroids, omega fatty acids and other such

suitable products of interest. Such products are useful in the context of biofuels, industrial and specialty chemicals, as intermediates used to make additional products, such as nutritional supplements, neutraceuticals, polymers, paraffin replacements, personal care products and pharmaceuticals.

[0092] Biofuel: A biofuel refers to any fuel that derives from a biological source. Biofuel can refer to one or more hydrocarbons, one or more alcohols (such as ethanol), one or more fatty esters, or a mixture thereof.

[0093] Hydrocarbon: The term generally refers to a chemical compound that consists of the elements carbon (C), hydrogen (H) and optionally oxygen (O). There are essentially three types of hydrocarbons, *e.g.*, aromatic hydrocarbons, saturated hydrocarbons and unsaturated hydrocarbons such as alkenes, alkynes, and dienes. The term also includes fuels, biofuels, plastics, waxes, solvents and oils. Hydrocarbons encompass biofuels, as well as plastics, waxes, solvents and oils.

[0094] The term "nutrient" refers to a component of a cell culture (e.g., one or more cells in a culture medium) or cell that can be used for growth and/or survival of the cell or cell culture. In some aspects, nutrients can be phosphorus (e.g., Phosphate, phosphite, phosphonates), sulfur (e.g., Sulfate, sulfite), nitrogen (e.g., Nitrate, nitrite, Ammonium or ammonia, urea), carbon (e.g., Inorganic carbon like carbon dioxide or bicarbonate/organic forms of carbon like sugars), iron (e.g., Iron salts), or trace metal components (e.g., manganese, cobalt, zinc, molybdenum, copper or boron); or combinations thereof. Included herein are methods for depleting one or more nutrients in the culture medium, e.g., to control growth of organisms such as undesirable microorganisms. It is contemplated that selection of an appropriate nutrient may depend on limiting one or more nutrients to contaminants in culture conditions and regulating such nutrient uptake in production hosts through genetic or process means. Accordingly, the selection of an appropriate nutrient in combination with regulation thereof is deemed within the scope of the invention.

[0095] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this present invention pertains. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice of the present invention and will be apparent to those of skill in the art. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

[0096] Throughout this specification and claims, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Nucleic Acid Sequences

[0097] The present invention provides isolated nucleic acid molecules for genes encoding enzymes, and variants thereof. Exemplary full-length nucleic acid sequences for genes encoding enzymes and the corresponding amino acid sequences are presented in the Table(s). The nucleic acid sequence can be preferably greater than 80%, 85%, 90%, 95%, 98%, 99%, 99.9% or even higher identity to the wild-type gene.

[0098] In another embodiment, the nucleic acid molecule of the present invention encodes a polypeptide having an amino acid sequence disclosed in the Table(s). Preferably, the nucleic acid molecule of the present invention encodes a polypeptide sequence of at least 50%, 60, 70%, 80%, 85%, 90% or 95% identity to the amino acid sequences shown in the Table(s)and the identity can even more preferably be 96%, 97%, 98%, 99%, 99.9% or even higher.

[0099] The present invention also provides nucleic acid molecules that hybridize under stringent conditions to the above-described nucleic acid molecules. As defined above, and as is well known in the art, stringent hybridizations are performed at about 25°C below the thermal melting point (T_m) for the specific DNA hybrid under a particular set of conditions, where the T_m is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. Stringent washing is performed at temperatures about 5°C lower than the T_m for the specific DNA hybrid under a particular set of conditions.

[00100] Nucleic acid molecules comprising a fragment of any one of the above-described nucleic acid sequences are also provided. These fragments preferably contain at least 20 contiguous nucleotides. More preferably the fragments of the nucleic acid sequences contain at least 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or even more contiguous nucleotides.

[00101] The nucleic acid sequence fragments of the present invention display utility in a variety of systems and methods. For example, the fragments may be used as probes in various hybridization techniques. Depending on the method, the target nucleic acid sequences may be either DNA or RNA. The target nucleic acid sequences may be fractionated (*e.g.*, by gel electrophoresis) prior to the hybridization, or the hybridization may be performed on samples in situ. One of skill in the art will appreciate that nucleic acid probes of known sequence find utility in determining chromosomal structure (*e.g.*, by Southern blotting) and in measuring gene expression (*e.g.*, by Northern blotting). In such

experiments, the sequence fragments are preferably detectably labeled, so that their specific hydridization to target sequences can be detected and optionally quantified. One of skill in the art will appreciate that the nucleic acid fragments of the present invention may be used in a wide variety of blotting techniques not specifically described herein.

[00102] It should also be appreciated that the nucleic acid sequence fragments disclosed herein also find utility as probes when immobilized on microarrays. Methods for creating microarrays by deposition and fixation of nucleic acids onto support substrates are well known in the art. Reviewed in *DNA Microarrays: A Practical Approach* (Practical Approach Series), Schena (ed.), Oxford University Press (1999) (ISBN: 0199637768); Nature Genet. 21(1)(suppl):1-60 (1999); *Microarray Biochip: Tools and Technology*, Schena (ed.), Eaton Publishing Company/BioTechniques Books Division (2000) (ISBN: 1881299376), the disclosures of which are incorporated herein by reference in their entireties. Analysis of, for example, gene expression using microarrays comprising nucleic acid sequence fragments, such as the nucleic acid sequence fragments disclosed herein, is a well-established utility for sequence fragments in the field of cell and molecular biology. Other uses for sequence fragments immobilized on microarrays are described in Gerhold *et al.*, *Trends Biochem. Sci.* 24:168-173 (1999) and Zweiger, *Trends Biotechnol.* 17:429-436 (1999); *DNA Microarrays: A Practical Approach* (Practical Approach Series), Schena (ed.), Oxford University Press (1999) (ISBN: 0199637768); Nature Genet. 21(1)(suppl):1-60 (1999); *Microarray Biochip: Tools and Technology*, Schena (ed.), Eaton Publishing Company/BioTechniques Books Division (2000) (ISBN: 1881299376), the disclosure of each of which is incorporated herein by reference in its entirety.

[00103] As is well known in the art, enzyme activities can be measured in various ways. For example, the pyrophosphorolysis of OMP may be followed spectroscopically (Grubmeyer *et al.*, (1993) *J. Biol. Chem.* 268:20299-20304). Alternatively, the activity of the enzyme can be followed using chromatographic techniques, such as by high performance liquid chromatography (Chung and Sloan, (1986) *J. Chromatogr.* 371:71-81). As another alternative the activity can be indirectly measured by determining the levels of product made from the enzyme activity. These levels can be measured with techniques including aqueous chloroform/methanol extraction as known and described in the art (*Cf.* M. Kates (1986) *Techniques of Lipidology; Isolation, analysis and identification of Lipids*. Elsevier Science Publishers, New York (ISBN: 0444807322)). More modern techniques include using gas chromatography linked to mass spectrometry (Niessen, W. M. A. (2001). *Current practice of gas chromatography--mass spectrometry*. New York, N.Y: Marcel Dekker. (ISBN:

0824704738)). Additional modern techniques for identification of recombinant protein activity and products including liquid chromatography-mass spectrometry (LCMS), high performance liquid chromatography (HPLC), capillary electrophoresis, Matrix-Assisted Laser Desorption Ionization time of flight-mass spectrometry (MALDI-TOF MS), nuclear magnetic resonance (NMR), near-infrared (NIR) spectroscopy, viscometry (Knothe, G (1997) *Am. Chem. Soc. Symp. Series*, 666: 172–208), titration for determining free fatty acids (Komers (1997) *Fett/Lipid*, 99(2): 52–54), enzymatic methods (Bailer (1991) *Fresenius J. Anal. Chem.* 340(3): 186), physical property-based methods, wet chemical methods, *etc.* can be used to analyze the levels and the identity of the product produced by the organisms of the present invention. Other methods and techniques may also be suitable for the measurement of enzyme activity, as would be known by one of skill in the art.

Vectors

[00104] Also provided are vectors, including expression vectors, which comprise the above nucleic acid molecules of the present invention, as described further herein. In a first embodiment, the vectors include the isolated nucleic acid molecules described above. In an alternative embodiment, the vectors of the present invention include the above-described nucleic acid molecules operably linked to one or more expression control sequences. The vectors of the instant invention may thus be used to express a polypeptide contributing to a carbon-based product of interest producing activity by a host cell.

[00105] Vectors useful for expression of nucleic acids in prokaryotes are well known in the art.

Isolated Polypeptides

[00106] According to another aspect of the present invention, isolated polypeptides (including muteins, allelic variants, fragments, derivatives, and analogs) encoded by the nucleic acid molecules of the present invention are provided. In one embodiment, the isolated polypeptide comprises the polypeptide sequence corresponding to a polypeptide sequence shown in the Table(s). In an alternative embodiment of the present invention, the isolated polypeptide comprises a polypeptide sequence at least 85% identical to a polypeptide sequence shown in the Table(s). Preferably the isolated polypeptide of the present invention has at least 50%, 60, 70%, 80%, 85%, 90%, 95%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%,

99.7%, 99.8%, 99.9% or even higher identity to a polypeptide sequence shown in the Table(s).

[00107] According to other embodiments of the present invention, isolated polypeptides comprising a fragment of the above-described polypeptide sequences are provided. These fragments preferably include at least 20 contiguous amino acids, more preferably at least 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or even more contiguous amino acids.

[00108] The polypeptides of the present invention also include fusions between the above-described polypeptide sequences and heterologous polypeptides. The heterologous sequences can, for example, include sequences designed to facilitate purification, *e.g.* histidine tags, and/or visualization of recombinantly-expressed proteins. Other non-limiting examples of protein fusions include those that permit display of the encoded protein on the surface of a phage or a cell, fusions to intrinsically fluorescent proteins, such as green fluorescent protein (GFP), and fusions to the IgG Fc region.

Host Cell Transformants

[00109] In another aspect of the present invention, host cells transformed with the nucleic acid molecules or vectors of the present invention, and descendants thereof, are provided. In some embodiments of the present invention, these cells carry the nucleic acid sequences of the present invention on vectors, which may but need not be freely replicating vectors. In other embodiments of the present invention, the nucleic acids have been integrated into the genome of the host cells.

[00110] In an alternative embodiment, the host cells of the present invention can be mutated by recombination with a disruption, deletion or mutation of the isolated nucleic acid of the present invention so that the activity of one or more enzyme(s) in the host cell is reduced or eliminated compared to a host cell lacking the mutation.

Selected or Engineered Microorganisms For the Production of Carbon-Based Products of Interest

[00111] Microorganism: Includes prokaryotic and eukaryotic microbial species from the Domains *Archaea, Bacteria* and *Eucarya*, the latter including yeast and filamentous fungi, protozoa, algae, or higher Protista. The terms "microbial cells" and "microbes" are used interchangeably with the term microorganism.

[00112] A variety of host organisms can be transformed to produce a product of interest. Photoautotrophic organisms include eukaryotic plants and algae, as well as prokaryotic

cyanobacteria, green-sulfur bacteria, green non-sulfur bacteria, purple sulfur bacteria, and purple non-sulfur bacteria.

[00113] Extremophiles are also contemplated as suitable organisms. Such organisms withstand various environmental parameters such as temperature, radiation, pressure, gravity, vacuum, desiccation, salinity, pH, oxygen tension, and chemicals. They include hyperthermophiles, which grow at or above 80°C such as *Pyrolobus fumarii*; thermophiles, which grow between 60-80°C such as *Synechococcus lividis*; mesophiles, which grow between 15-60°C and psychrophiles, which grow at or below 15°C such as *Psychrobacter* and some insects. Radiation tolerant organisms include *Deinococcus radiodurans*. Pressure-tolerant organisms include piezophiles, which tolerate pressure of 130 MPa. Weight-tolerant organisms include barophiles. Hypergravity (*e.g.*,, >1g) hypogravity (*e.g.*, <1g) tolerant organisms are also contemplated. Vacuum tolerant organisms include tardigrades, insects, microbes and seeds. Dessicant tolerant and anhydrobiotic organisms include xerophiles such as *Artemia salina*; nematodes, microbes, fungi and lichens. Salt-tolerant organisms include halophiles (*e.g.*, 2-5 M NaCl) *Halobacteriacea* and *Dunaliella salina*. pH-tolerant organisms include alkaliphiles such as *Natronobacterium*, *Bacillus firmus* OF4, *Spirulina spp.* (*e.g.*, pH > 9) and acidophiles such as *Cyanidium caldarium*, *Ferroplasma sp.* (*e.g.*, low pH). Anaerobes, which cannot tolerate O_2 such as *Methanococcus jannaschii*; microaerophils, which tolerate some O_2 such as *Clostridium* and aerobes, which require O_2 are also contemplated. Gas-tolerant organisms, which tolerate pure CO_2 include *Cyanidium caldarium* and metal tolerant organisms include metalotolerants such as *Ferroplasma acidarmanus* (*e.g.*, Cu, As, Cd, Zn), *Ralstonia* sp. CH34 (*e.g.*, Zn, Co, Cd, Hg, Pb). Gross, Michael. *Life on the Edge: Amazing Creatures Thriving in Extreme Environments*. New York: Plenum (1998) and Seckbach, J. "Search for Life in the Universe with Terrestrial Microbes Which Thrive Under Extreme Conditions." In Cristiano Batalli Cosmovici, Stuart Bowyer, and Dan Wertheimer, eds., *Astronomical and Biochemical Origins and the Search for Life in the Universe*, p. 511. Milan: Editrice Compositori (1997).

[00114] Plants include but are not limited to the following genera: *Arabidopsis, Beta, Glycine, Jatropha, Miscanthus, Panicum, Phalaris, Populus, Saccharum, Salix, Simmondsia* and *Zea.*

[00115] Algae and cyanobacteria include but are not limited to the following genera: *Acanthoceras, Acanthococcus, Acaryochloris, Achnanthes, Achnanthidium, Actinastrum, Actinochloris, Actinocyclus, Actinotaenium, Amphichrysis, Amphidinium, Amphikrikos, Amphipleura, Amphiprora, Amphithrix, Amphora, Anabaena, Anabaenopsis, Aneumastus,*

Ankistrodesmus, Ankyra, Anomoeoneis, Apatococcus, Aphanizomenon, Aphanocapsa, Aphanochaete, Aphanothece, Apiocystis, Apistonema, Arthrodesmus, Artherospira, Ascochloris, Asterionella, Asterococcus, Audouinella, Aulacoseira, Bacillaria, Balbiania, Bambusina, Bangia, Basichlamys, Batrachospermum, Binuclearia, Bitrichia, Blidingia, Botrdiopsis, Botrydium, Botryococcus, Botryosphaerella, Brachiomonas, Brachysira, Brachytrichia, Brebissonia, Bulbochaete, Bumilleria, Bumilleriopsis, Caloneis, Calothrix, Campylodiscus, Capsosiphon, Carteria, Catena, Cavinula, Centritractus, Centronella, Ceratium, Chaetoceros, Chaetochloris, Chaetomorpha, Chaetonella, Chaetonema, Chaetopeltis, Chaetophora, Chaetosphaeridium, Chamaesiphon, Chara, Characiochloris, Characiopsis, Characium, Charales, Chilomonas, Chlainomonas, Chlamydoblepharis, Chlamydocapsa, Chlamydomonas, Chlamydomonopsis, Chlamydomyxa, Chlamydonephris, Chlorangiella, Chlorangiopsis, Chlorella, Chlorobotrys, Chlorobrachis, Chlorochytrium, Chlorococcum, Chlorogloea, Chlorogloeopsis, Chlorogonium, Chlorolobion, Chloromonas, Chlorophysema, Chlorophyta, Chlorosaccus, Chlorosarcina, Choricystis, Chromophyton, Chromulina, Chroococcidiopsis, Chroococcus, Chroodactylon, Chroomonas, Chroothece, Chrysamoeba, Chrysapsis, Chrysidiastrum, Chrysocapsa, Chrysocapsella, Chrysochaete, Chrysochromulina, Chrysococcus, Chrysocrinus, Chrysolepidomonas, Chrysolykos, Chrysonebula, Chrysophyta, Chrysopyxis, Chrysosaccus, Chrysophaerella, Chrysostephanosphaera, Clodophora, Clastidium, Closteriopsis, Closterium, Coccomyxa, Cocconeis, Coelastrella, Coelastrum, Coelosphaerium, Coenochloris, Coenococcus, Coenocystis, Colacium, Coleochaete, Collodictyon, Compsogonopsis, Compsopogon, Conjugatophyta, Conochaete, Coronastrum, Cosmarium, Cosmioneis, Cosmocladium, Crateriportula, Craticula, Crinalium, Crucigenia, Crucigeniella, Cryptoaulax, Cryptomonas, Cryptophyta, Ctenophora, Cyanodictyon, Cyanonephron, Cyanophora, Cyanophyta, Cyanothece, Cyanothomonas, Cyclonexis, Cyclostephanos, Cyclotella, Cylindrocapsa, Cylindrocystis, Cylindrospermum, Cylindrotheca, Cymatopleura, Cymbella, Cymbellonitzschia, Cystodinium Dactylococcopsis, Debarya, Denticula, Dermatochrysis, Dermocarpa, Dermocarpella, Desmatractum, Desmidium, Desmococcus, Desmonema, Desmosiphon, Diacanthos, Diacronema, Diadesmis, Diatoma, Diatomella, Dicellula, Dichothrix, Dichotomococcus, Dicranochaete, Dictyochloris, Dictyococcus, Dictyosphaerium, Didymocystis, Didymogenes, Didymosphenia, Dilabifilum, Dimorphococcus, Dinobryon, Dinococcus, Diplochloris, Diploneis, Diplostauron, Distrionella, Docidium, Draparnaldia, Dunaliella, Dysmorphococcus, Ecballocystis, Elakatothrix, Ellerbeckia, Encyonema, Enteromorpha, Entocladia, Entomoneis,

Entophysalis, Epichrysis, Epipyxis, Epithemia, Eremosphaera, Euastropsis, Euastrum, Eucapsis, Eucocconeis, Eudorina, Euglena, Euglenophyta, Eunotia, Eustigmatophyta, Eutreptia, Fallacia, Fischerella, Fragilaria, Fragilariforma, Franceia, Frustulia, Curcilla, Geminella, Genicularia, Glaucocystis, Glaucophyta, Glenodiniopsis, Glenodinium, Gloeocapsa, Gloeochaete, Gloeochrysis, Gloeococcus, Gloeocystis, Gloeodendron, Gloeomonas, Gloeoplax, Gloeothece, Gloeotila, Gloeotrichia, Gloiodictyon, Golenkinia, Golenkiniopsis, Gomontia, Gomphocymbella, Gomphonema, Gomphosphaeria, Gonatozygon, Gongrosia, Gongrosira, Goniochloris, Gonium, Gonyostomum, Granulochloris, Granulocystopsis, Groenbladia, Gymnodinium, Gymnozyga, Gyrosigma, Haematococcus, Hafniomonas, Hallassia, Hammatoidea, Hannaea, Hantzschia, Hapalosiphon, Haplotaenium, Haptophyta, Haslea, Hemidinium, Hemitoma, Heribaudiella, Heteromastix, Heterothrix, Hibberdia, Hildenbrandia, Hillea, Holopedium, Homoeothrix, Hormanthonema, Hormotila, Hyalobrachion, Hyalocardium, Hyalodiscus, Hyalogonium, Hyalotheca, Hydrianum, Hydrococcus, Hydrocoleum, Hydrocoryne, Hydrodictyon, Hydrosera, Hydrurus, Hyella, Hymenomonas, Isthmochloron, Johannesbaptistia, Juranyiella, Karayevia, Kathablepharis, Katodinium, Kephyrion, Keratococcus, Kirchneriella, Klebsormidium, Kolbesia, Koliella, Komarekia, Korshikoviella, Kraskella, Lagerheimia, Lagynion, Lamprothamnium, Lemanea, Lepocinclis, Leptosira, Lobococcus, Lobocystis, Lobomonas, Luticola, Lyngbya, Malleochloris, Mallomonas, Mantoniella, Marssoniella, Martyana, Mastigocoleus, Gastogloia, Melosira, Merismopedia, Mesostigma, Mesotaenium, Micractinium, Micrasterias, Microchaete, Microcoleus, Microcystis, Microglena, Micromonas, Microspora, Microthamnion, Mischococcus, Monochrysis, Monodus, Monomastix, Monoraphidium, Monostroma, Mougeotia, Mougeotiopsis, Myochloris, Myromecia, Myxosarcina, Naegeliella, Nannochloris, Nautococcus, Navicula, Neglectella, Neidium, Nephroclamys, Nephrocytium, Nephrodiella, Nephroselmis, Netrium, Nitella, Nitellopsis, Nitzschia, Nodularia, Nostoc, Ochromonas, Oedogonium, Oligochaetophora, Onychonema, Oocardium, Oocystis, Opephora, Ophiocytium, Orthoseira, Oscillatoria, Oxyneis, Pachycladella, Palmella, Palmodictyon, Pnadorina, Pannus, Paralia, Pascherina, Paulschulzia, Pediastrum, Pedinella, Pedinomonas, Pedinopera, Pelagodictyon, Penium, Peranema, Peridiniopsis, Peridinium, Peronia, Petroneis, Phacotus, Phacus, Phaeaster, Phaeodermatium, Phaeophyta, Phaeosphaera, Phaeothamnion, Phormidium, Phycopeltis, Phyllariochloris, Phyllocardium, Phyllomitas, Pinnularia, Pitophora, Placoneis, Planctonema, Planktosphaeria, Planothidium, Plectonema, Pleodorina, Pleurastrum, Pleurocapsa, Pleurocladia, Pleurodiscus, Pleurosigma, Pleurosira, Pleurotaenium,

Pocillomonas, Podohedra, Polyblepharides, Polychaetophora, Polyedriella, Polyedriopsis, Polygoniochloris, Polyepidomonas, Polytaenia, Polytoma, Polytomella, Porphyridium, Posteriochromonas, Prasinochloris, Prasinocladus, Prasinophyta, Prasiola, Prochlorphyta, Prochlorothrix, Protoderma, Protosiphon, Provasoliella, Prymnesium, Psammodictyon, Psammothidium, Pseudanabaena, Pseudenoclonium, Psuedocarteria, Pseudochate, Pseudocharacium, Pseudococcomyxa, Pseudodictyosphaerium, Pseudokephyrion, Pseudoncobyrsa, Pseudoquadrigula, Pseudosphaerocystis, Pseudostaurastrum, Pseudostaurosira, Pseudotetrastrum, Pteromonas, Punctastruata, Pyramichlamys, Pyramimonas, Pyrrophyta, Quadrichloris, Quadricoccus, Quadrigula, Radiococcus, Radiofilum, Raphidiopsis, Raphidocelis, Raphidonema, Raphidophyta, Peimeria, Rhabdoderma, Rhabdomonas, Rhizoclonium, Rhodomonas, Rhodophyta, Rhoicosphenia, Rhopalodia, Rivularia, Rosenvingiella, Rossithidium, Roya, Scenedesmus, Scherffelia, Schizochlamydella, Schizochlamys, Schizomeris, Schizothrix, Schroederia, Scolioneis, Scotiella, Scotiellopsis, Scourfieldia, Scytonema, Selenastrum, Selenochloris, Sellaphora, Semiorbis, Siderocelis, Diderocystopsis, Dimonsenia, Siphononema, Sirocladium, Sirogonium, Skeletonema, Sorastrum, Spermatozopsis, Sphaerellocystis, Sphaerellopsis, Sphaerodinium, Sphaeroplea, Sphaerozosma, Spiniferomonas, Spirogyra, Spirotaenia, Spirulina, Spondylomorum, Spondylosium, Sporotetras, Spumella, Staurastrum, Stauerodesmus, Stauroneis, Staurosira, Staurosirella, Stenopterobia, Stephanocostis, Stephanodiscus, Stephanoporos, Stephanosphaera, Stichococcus, Stichogloea, Stigeoclonium, Stigonema, Stipitococcus, Stokesiella, Strombomonas, Stylochrysalis, Stylodinium, Styloyxis, Stylosphaeridium, Surirella, Sykidion, Symploca, Synechococcus, Synechocystis, Synedra, Synochromonas, Synura, Tabellaria, Tabularia, Teilingia, Temnogametum, Tetmemorus, Tetrachlorella, Tetracyclus, Tetradesmus, Tetraedriella, Tetraedron, Tetraselmis, Tetraspora, Tetrastrum, Thalassiosira, Thamniochaete, Thorakochloris, Thorea, Tolypella, Tolypothrix, Trachelomonas, Trachydiscus, Trebouxia, Trentepholia, Treubaria, Tribonema, Trichodesmium, Trichodiscus, Trochiscia, Tryblionella, Ulothrix, Uroglena, Uronema, Urosolenia, Urospora, Uva, Vacuolaria, Vaucheria, Volvox, Volvulina, Westella, Woloszynskia, Xanthidium, Xanthophyta, Xenococcus, Zygnema, Zygnemopsis, and *Zygonium*. Cyanobacteria include members of the genus *Chamaesiphon, Chroococcus, Cyanobacterium, Cyanobium, Cyanothece, Dactylococcopsis, Gloeobacter, Gloeocapsa, Gloeothece, Microcystis, Prochlorococcus, Prochloron, Synechococcus, Synechocystis, Cyanocystis, Dermocarpella, Stanieria, Xenococcus, Chroococcidiopsis, Myxosarcina, Arthrospira, Borzia, Crinalium, Geitlerinemia, Leptolyngbya, Limnothrix, Lyngbya,*

Microcoleus, Oscillatoria, Planktothrix, Prochiorothrix, Pseudanabaena, Spirulina, Starria, Symploca, Trichodesmium, Tychonema, Anabaena, Anabaenopsis, Aphanizomenon, Cyanospira, Cylindrospermopsis, Cylindrospermum, Nodularia, Nostoc, Scylonema, Calothrix, Rivularia, Tolypothrix, Chlorogloeopsis, Fischerella, Geitieria, Iyengariella, Nostochopsis, Stigonema and *Thermosynechococcus*.

[00116] Green non-sulfur bacteria include but are not limited to the following genera: *Chloroflexus, Chloronema, Oscillochloris, Heliothrix, Herpetosiphon, Roseiflexus*, and *Thermomicrobium*.

[00117] Green sulfur bacteria include but are not limited to the following genera:

[00118] *Chlorobium, Clathrochloris*, and *Prosthecochloris*.

[00119] Purple sulfur bacteria include but are not limited to the following genera: *Allochromatium, Chromatium, Halochromatium, Isochromatium, Marichromatium, Rhodovulum, Thermochromatium, Thiocapsa, Thiorhodococcus*, and *Thiocystis*,

[00120] Purple non-sulfur bacteria include but are not limited to the following genera: *Phaeospirillum, Rhodobaca, Rhodobacter, Rhodomicrobium, Rhodopila, Rhodopseudomonas, Rhodothalassium, Rhodospirillum, Rodovibrio*, and *Roseospira*.

[00121] Aerobic chemolithotrophic bacteria include but are not limited to nitrifying bacteria such as *Nitrobacteraceae* sp., *Nitrobacter* sp., *Nitrospina* sp., *Nitrococcus* sp., *Nitrospira* sp., *Nitrosomonas* sp., *Nitrosococcus* sp., *Nitrosospira* sp., *Nitrosolobus* sp., *Nitrosovibrio* sp.; colorless sulfur bacteria such as, *Thiovulum* sp., *Thiobacillus* sp., *Thiomicrospira* sp., *Thiosphaera* sp., *Thermothrix* sp.; obligately chemolithotrophic hydrogen bacteria such as *Hydrogenobacter* sp., iron and manganese-oxidizing and/or depositing bacteria such as *Siderococcus* sp., and magnetotactic bacteria such as *Aquaspirillum* sp.

[00122] Archaeobacteria include but are not limited to methanogenic archaeobacteria such as *Methanobacterium* sp., *Methanobrevibacter* sp., *Methanothermus* sp., *Methanococcus* sp., *Methanomicrobium* sp., *Methanospirillum* sp., *Methanogenium* sp., *Methanosarcina* sp., *Methanolobus* sp., *Methanothrix* sp., *Methanococcoides* sp., *Methanoplanus* sp.; extremely thermophilic S-Metabolizers such as *Thermoproteus* sp., *Pyrodictium* sp., *Sulfolobus* sp., *Acidianus* sp. and other microorganisms such as, *Bacillus subtilis, Saccharomyces cerevisiae, Streptomyces* sp., *Ralstonia* sp., *Rhodococcus* sp., *Corynebacteria* sp., *Brevibacteria* sp., *Mycobacteria* sp., and oleaginous yeast.

[00123] Preferred organisms for the manufacture of carbon-based products of interest according to the methods discloused herein include: *Arabidopsis thaliana, Panicum virgatum, Miscanthus giganteus*, and *Zea mays* (plants); *Botryococcus braunii, Chlamydomonas*

reinhardtii and *Dunaliela salina* (algae); *Synechococcus* sp PCC 7002, *Synechococcus* sp. PCC 7942, *Synechocystis* sp. PCC 6803, *Thermosynechococcus elongatus* BP-1 (cyanobacteria); *Chlorobium tepidum* (green sulfur bacteria), *Chloroflexus auranticus* (green non-sulfur bacteria); *Chromatium tepidum* and *Chromatium vinosum* (purple sulfur bacteria); *Rhodospirillum rubrum, Rhodobacter capsulatus*, and *Rhodopseudomonas palusris* (purple non-sulfur bacteria).

[00124] Yet other suitable organisms include synthetic cells or cells produced by synthetic genomes as described in Venter *et al.* US Pat. Pub. No. 2007/0264688, and cell-like systems or synthetic cells as described in Glass *et al.* US Pat. Pub. No. 2007/0269862.

[00125] Still, other suitable organisms include microorganisms that can be engineered to fix carbon dioxide bacteria such as *Escherichia coli*, *Acetobacter aceti*, *Bacillus subtilis*, yeast and fungi such as *Clostridium ljungdahlii*, *Clostridium thermocellum*, *Penicillium chrysogenum*, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pseudomonas fluorescens*, or *Zymomonas mobilis*.

[00126] A suitable organism for selecting or engineering is capable of autotrophic fixation of CO_2 to products. This would cover photosynthesis and methanogenesis. Acetogenesis, encompassing the three types of CO_2 fixation; Calvin cycle, acetyl-CoA pathway and reductive TCA pathway is also covered. The capability to use carbon dioxide as the sole source of cell carbon (autotrophy) is found in almost all major groups of prokaryotes. The CO_2 fixation pathways differ between groups, and there is no clear distribution pattern of the four presently-known autotrophic pathways. See, *e.g.*, Fuchs, G. 1989. *Alternative pathways of autotrophic CO_2 fixation*, p. 365-382. *In* H. G. Schlegel, and B. Bowien (ed.), *Autotrophic bacteria*. Springer-Verlag, Berlin, Germany. The reductive pentose phosphate cycle (Calvin-Bassham-Benson cycle) represents the CO_2 fixation pathway in almost all aerobic autotrophic bacteria, for example, the cyanobacteria.

[00127] Carbon-based product of interest production via engineered cyanobacteria, *e.g.*, a *Synechococcus* or *Thermosynechococcus* species, is preferred. Other preferred organisms include *Synechocystis*, *Klebsiella oxytoca*, *Escherichia coli* or *Saccharomyces cerevisiae*. Other prokaryotic, archaea and eukaryotic host cells are also encompassed within the scope of the present invention.

[00128] In some aspects, carbon-based product of interest production via a photosynthetic organism can be carried out using the compositions, materials, and methods described in: PCT/US2009/035937 (filed March 3, 2009); and PCT/US2009/055949 (filed September 3, 2009); each of which is herein incorporated by reference in its entirety, for all purposes.

Carbon-Based Products of Interest: Hydrocarbons & Alcohols

[00129] In various embodiments of the invention, desired hydrocarbons and/or alcohols of certain chain length or a mixture thereof can be produced. In certain aspects, the host cell produces at least one of the following carbon-based products of interest: alkanes such as heptane, nonane, tridecane, pentadecane, and/or undecane and/or alcohols such as ethanol. In other aspects, the carbon chain length ranges from C_2 to C_{20}, e.g., C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20}. Accordingly, the invention provides production of various carbon chain lengths suitable for use as fuels & chemicals.

[00130] In preferred aspects, the methods provide culturing host cells for direct product secretion for easy recovery without the need to extract biomass. These carbon-based products of interest are secreted directly into the medium. Since the invention enables production of various defined chain length of hydrocarbons and alcohols, the secreted products are easily recovered or separated. The products of the invention, therefore, can be used directly or used with minimal processing.

Fuel Compositions

[00131] In various embodiments, compositions produced by the methods of the invention are used as fuels. Such fuels comply with ASTM standards, for instance, standard specifications for diesel fuel oils D 975-09b, and Jet A, Jet A-1 and Jet B as specified in ASTM Specification D. 1655-68. Fuel compositions may require blending of several products to produce a uniform product. The blending process is relatively straightforward, but the determination of the amount of each component to include in a blend is much more difficult. Fuel compositions may, therefore, include aromatic and/or branched hydrocarbons, for instance, 75% saturated and 25% aromatic, wherein some of the saturated hydrocarbons are branched and some are cyclic. Preferably, the methods of the invention produce an array of hydrocarbons, such as C_2-C_{17} or C_{10}-C_{15} to alter cloud point. Furthermore, the compositions may comprise fuel additives, which are used to enhance the performance of a fuel or engine. For example, fuel additives can be used to alter the freezing/gelling point, cloud point, lubricity, viscosity, oxidative stability, ignition quality, octane level, and flash point. Fuels compositions may also comprise, among others, antioxidants, static dissipater, corrosion inhibitor, icing inhibitor, biocide, metal deactivator and thermal stability improver.

[00132] In addition to many environmental advantages of the invention such as CO_2 conversion and renewable source, other advantages of the fuel compositions disclosed herein

include low sulfur content, low emissions, being free or substantially free of alcohol and having high cetane number.

Example 1: Increased rate of phosphate uptake in ΔphoU ethanologen with a maintained rate of ethanol production.

[00133] Strain construction:

[00134] The construction of urea-repressible ethanologen JCC1581 was detailed in patent application PCT/US12/71250, herein incorporated by reference. JCC5048 was prepared in the same manner with the sole difference that the P(cum02) promoter (Table A) regulates the expression of the *pdc-adh* operon instead of P(nir07). To obtain the *phoU* knockout, a chromosomal targeting vector was constructed containing 500 bp of upstream and downstream sequence homology to *phoU* allowing insertion of an erythromycin marker in place of the *phoU* gene (SYNPCC7002_A1708; Table A). The sequence and annotation of this plasmid (pJB3730) is provided in Table A. This plasmid was naturally transformed into JCC138 using a standard cyanobacterial transformation and segregation protocol. The genotypes of the two strains of cyanobacteria referenced in this example are provided in Table 1.

Table 1. Joule Culture Collection (JCC) numbers of the *Synechococcus* sp. PCC 7002-based strains investigated for phosphate uptake, ethanol production, and bioburden/contamination control

Strain	Genotype
JCC138	*Synechococcus* sp. PCC 7002
JCC5048	JCC138 pAQ3::P(cum02)-pdc_{Zmo}-*adhAM* pAQ7(Δ*ldh*)::P(cI)-*adhAM*
JCC5473	JCC5048 Δ*phoU*

[00135] Culture conditions and sampling:

[00136] A clonal culture of two ethanologen strains described in table 1 was grown in A+ medium supplemented with 15 mM urea. These strains were incubated at 37 °C at 150 rpm in 2 % CO_2-enriched air at ~100 µmol photons m^{-2} s^{-1} in a Multitron II (Infors) shaking photoincubator. These cultures were then used to inoculate 30 ml cultures in 125 ml PETG flasks to an OD_{730} = 0.5 in JB3.0 medium supplemented with either 3 or 4 mM urea and

containing 0.25x or 1x the amount of phosphate contained in JB3.0 base medium. JB3.0 medium (pH ~8.2) consists of 18.0 g/l sodium chloride, 2.5 g/l magnesium sulfate heptahydrate, 4.0 g/l sodium nitrate, 1.0 g/l Tris, 0.6 g/l potassium chloride, 0.14 g/l calcium chloride (anhydrous), 0.2 g/l potassium phosphate monobasic, 34.3 mg/l boric acid, 29.4 mg/l EDTA (disodium salt dihydrate), 21.1 mg/l EDTA iron (III) sodium salt, 4.3 mg/l manganese chloride tetrahydrate, 315.0 µg/l zinc chloride, 30.0 µg/l molybdenum (VI) oxide, 12.2 µg/l cobalt (II) chloride hexahydrate, 10.0 µg/l vitamin B_{12}, and 3.0 µg/l copper (II) sulfate pentahydrate. The cultures were incubated in a Multitron II (Infors) shaking photoincubator under the same conditions as the inoculum culture. Samples were taken for growth by taking OD_{730} measurements and supernatant samples were taken to quantify the phosphate and ethanol concentrations in the medium.

[00137] Quantification of phosphate and ethanol:

[00138] The commercially-available phosphate assay kit from Abcam (ab65622) was used following the provided protocol with the following exceptions. A phosphate calibration curve was prepared in JB3.0 medium between 0-60 µM (5-60 µM is the linear range of the assay in this medium, limit of detection ~1 µM) and culture supernatant dilutions were carried out in phosphate-free medium when necessary for quantification. 100 µl of sample was added and 10 µl of phosphate reagent to each well in the 96-well plate. The room temperature incubation time period was 1 hour instead of 30 min.

[00139] The ethanol quantification and workup was performed as previously described (PCT/US12/71250). Briefly, the ethanol titre plots indicate the actual ethanol concentration in the flasks, and the cumulative ethanol plots indicate the total amount of ethanol produced by the strain under each condition (ethanol concentrations are adjusted to account for loss due to stripping from the flasks).

[00140] Results:

[00141] The growth of JCC5048 and JCC5473 was similar in JB3.0 medium containing 0.25x phosphate (figure 1), but the phosphate uptake rate by JCC5473 was much faster (figure 2). The rate of phosphate uptake by JCC5473 was approximately 9-fold faster than JCC5048 (table 2), leading to phosphate depletion by the 4 hour timepoint whereas JCC5048 took over 24 hours to accomplish phosphate depletion in the medium. With 1x phosphate present, JCC5473 grew comparably to JCC5048 (figure 3) and consumed all the phosphate by the 24 h timepoint (figure 4) but JCC5048 did not until day 6 (if ethanol production was not induced) or until day 8 (if ethanol production was induced). Through day 10, the ethanol production of JCC5473 matches that of JCC5048 (figure 5).

Table 2. Phosphate uptake rates of the ethanologen strains	
Strain	Phosphate uptake rate (μM h^{-1})
JCC5048	8.9
JCC5473	79.5

Example 2: Contaminated JCC5473 cultures produce ethanol at same rate as clean cultures.

[00142] Culture conditions and sampling:

[00143] JCC5048 and JCC5473 cultures were used to inoculate 30 ml cultures in 125 ml PETG flasks to an OD_{730} = 0.5 in JB3.0 base medium amended with 3 mM urea and containing 0.5x the amount of potassium phosphate contained in JB 3.0 medium (see example 1 for culturing conditions). At the beginning of the experiment, three strains of ethanol-catabolizing heterotrophs (Table 3) were added to concentration of 10 colony-forming units (CFUs) per ml to one flask for both strains, and 10^3 CFU/ml was added to another flask of JCC5473. One flask for each strain received no addition of contamination for ethanol production rate comparison. Cultures were monitored for growth by taking OD_{730} measurements. Culture supernatant samples were used to quantify the ethanol and phosphate concentrations.

Table 3. The three-strain contaminant panel used for the ethanol catabolism studies.	
Contaminant panel #	Strain
1	*Acinetobacter baylyi* ADP1
2	*Bacillus thuringiensis*
3	*Pseudomonas stutzeri*

[00144] Results:

[00145] JCC5473 and JCC5048 grew similarly and JCC5473 was able to sustain the same rate of ethanol production as the control flasks (figures 6-7, Table 4) even in the presence of a high concentration of contaminant cells at the beginning of the experiment (10^3 CFU/ml). However, JCC5048 was not able to produce any significant amount ethanol even with the lower starting contaminant inoculation of 10 CFU/ml (figure 7).

Table 4. The ethanol production rates of the JCC5473 and JCC5048 cultures

		Day 4 -> 9	
Strain	Contaminant CFU/ml (T=0)	EtOH production mg L^{-1} h^{-1}	Residuals R^2
JCC5473	10	24	0.9946
	10^3	25	0.9991
	no contaminants (clean)	24	0.9839
JCC5048	10	0	0.8747
	no contaminants (clean)	24	0.9929

Example 3: Improved ethanol production timespan of JCC5473 supplemented with acetate

[00146] Culture conditions and sampling:

[00147] JCC5473 was used to inoculate duplicate cultures in 125 ml flasks to an OD_{730} = 0.5 in JB3.0 base medium amended with 3 mM urea (see example 1 for culturing conditions). On day 2, 20 µM of cumate was added to both flasks and one flask received a 10 mM sodium acetate addition (final concentration). Cultures were monitored for growth by taking OD_{730} measurements and culture supernatant samples were taken for ethanol quantification.

[00148] Results:

[00149] In the presence of 10 mM acetate, the ethanol production timespan of JCC5473 was extended by approximately 7 days leading to a greater than 2-fold yield of ethanol (figure 8). The growth of JCC5473 was superior with 10 mM acetate, as the culture was able to achieve an OD_{730} of ~9 compared to an OD_{730} of ~7 for the culture without acetate addition (figure 9).

Example 4: Phosphate loading of JCC5473

[00150] Culture conditions and sampling:

[00151] JCC5473 was used to inoculate 30 ml flasks to an OD_{730} = 0.5 in JB3.0 base medium containing either 1x, 2x, 3x or 4x the phosphate level of JB3.0. Cultures were monitored for growth by taking OD_{730} measurements and culture supernatant samples were taken for phosphate quantification. The culture containing 4x phosphate was washed with JB3.0 lacking phosphate to remove residual phosphate. The above cultures were used to inoculate duplicate 30ml flasks to an OD_{730} = 0.5 in JB3.0 base medium containing 0x

phosphate. On day two, 20 μM cumate was added one of the flasks, and cultures were monitored for growth by taking OD_{730} measurements and culture supernatant samples were taken for ethanol quantification.

[00152] Results:

[00153] JCC5473 was capable of taking up greater than 3x the phosphate normally present in JB3.0 (figure 10). As the biomass accumulation or phosphate uptake rate was unaffected by increased phosphate uptake (figure 10-11), JCC5473 can continue to accumulate phosphate if it is present in the medium. The cells with higher intracellular phosphate concentrations were capable of growing to a higher OD_{730} (figure 12) in media lacking phosphate, indicating that JCC5473 is capable of utilizing increased stores of phosphate. The JCC5473 cultures that had been incubated with 2x or higher phosphate were also able to produce 6-fold more ethanol in medium lacking phosphate than the JCC5473 culture incubated in 1x phosphate (figure 13), despite displaying similar growth after induction (figure 14).

Example 5: Improved ethanol production timespan of Δ*phoU* strains via expression of a catalase

[00154] Strain construction:

[00155] The construction of urea-repressible ethanologen JCC3351 expressing a catalase (*katG*) was detailed in the patent application PCT/US2012/071250, herein incorporated by reference. JCC5290 was prepared in the same manner as JCC3351 with the sole difference that P(cum02) promoter (see example 1) regulates the expression of the pdc-adh operon instead of P(nir07). The Δ*phoU* derivitive of JCC5290 was prepared as described in example 1 to yield JCC5476. The genotypes of these two strains are provided in table 5a.

Table 5a. Joule Culture Collection (JCC) numbers of the *Synechococcus* sp. PCC 7002-based strains investigated for phosphate uptake and ethanol production.

Strain	Genotype
JCC138	*Synechococcus* sp. PCC 7002
JCC5290	JCC138 pAQ3::P(cum02)-pdc_{zmo}-*adhAM* pAQ7(Δ*ldh*)::P(cI)-*adhAM* chrom::P(cpcB)-*katG*
JCC5476	JCC5290 Δ*phoU*

[00156] Culture conditions and sampling:

[00157] JCC5290, JCC5473 and JCC5476 were used to inoculate duplicate cultures in 125 ml flasks to an OD_{730} = 0.5 in JB3.0 base medium amended with 3 mM urea (see example 1 for culturing conditions). On day 2, 20 µM of cumate was added to all flasks and one of the flasks for each strain received a 10 mM sodium acetate addition (final concentration). Cultures were monitored for growth by taking OD_{730} measurements and culture supernatant samples were taken for ethanol quantification and phosphate uptake.

[00158] Results:

[00159] JCC5476 was able to produce ethanol for the same time period as JCC5290, and acetate addition did not extend the longevity of ethanol production (Figure 15). Therefore, expression of *katG* appears to mitigate the negative impact of the *phoU* knockout, indicating that the shortened lifespan of JCC5473 may be due to peroxide generation. Ethanol production was not enhanced by acetate addition to JCC5290 and JCC5476, but those cultures with acetate did reach a higher cell density (Figure 16). JCC5476 was able to remove all phosphate from the media by the day 1 timepoint (Figure 17), showing that the strain maintains a high rate of phosphate uptake with katG expression.

Example 6: Bioburden control outdoors using phosphate loading

[00160] Culture conditions and sampling:

[00161] JCC5048 and JCC5473 were grown in JB3.0 and JB3.0 with 3x phosphate. Cultures were harvested following depletion of JCC5473 and used to inoculate outdoor photobioreactors to the optical density indicated in JB3.0 with the phosphate levels indicated below. The photobioreactor setup utilized a 50 foot length of clear flex 70-1 culture tubing, with an inner diameter of 0.75 inches, and a tube wall of 0.125 inches. The reactor was sparged with 2% CO_2 at a rate of 0.80 liters per minute, circulated with a Watson Marlow 720u pump set at 180 revolutions per minute, and operated at pH of 7.2±.2 and 35°C. Average and peak solar insolation levels of 822 and 2228 uE/m2/s were observed respectively. At the time indicated by the arrow below, 5 µM cumate was added to the reactors. At T=44 hours *Pseudomonas stutzeri* was added to the indicated reactor at a concentration of 0.1 CFU/ml.

[00162] Results:

[00163] JCC5473 was capable of using solely internal phosphate stores such that growth (figure 18) and ethanol productivity (figure 19) matched that of the culture with additional phosphate input. Additionally, the contaminated culture of JCC5473 was capable of

controlling contamination throughout the run such that productivity was indistinguishable from that of the axenic JCC5473 reactor.

Example 6: Temporal control of phosphate uptake response via regulated expression of phoU

[00164] Strain construction

Table 5b. Joule Culture Collection (JCC) numbers of the *Synechococcus* sp. PCC 7002-based strains investigated for phosphate uptake and ethanol production

Strain	Genotype
JCC138	*Synechococcus* sp. PCC 7002
JCC5290	JCC138 pAQ3::P(cum02)-pdc_{zmo}-*adhAM* pAQ7(Δ*ldh*)::P(cI)-*adhAM* chrom::P(cpcB)-*katG*
JCC5476	JCC5290 Δ*phoU*
JCC6082	JCC5476 UHR_phoU::P(BreO2.1_theoRS)-*phoU* P(kanR)-*bla*::DHR_phoU

[00165] Culture conditions and sampling:

[00166] Cultures were inoculated into phosphate replete or depleted media with or without deoxycholate (DOC) as indicated, and supernatant samples were assessed for alkaline phosphatase (AP) activity. Samples were added at 1:1 ratio into a solution of 1 mg/L para-nitrophenylphosphate in 100mM Trizma buffer pH 9.0. Samples were incubated for 30 minutes and the absorbance at 405 nm of each sample was determined, and normalized to the JCC5048 replete absorbance.

[00167] Results

[00168] JCC5048 shows AP activity, but only when depleted for phosphate. JCC5473 shows AP activity independent of the phosphate concentration in the media. JCC6082 shows AP activity independent of phosphate concentration when repressed for *phoU*, but only in the absence of phosphate when induced with for *phoU* expression with the addition of DOC (table 6). Together this indicates that sufficient control of *phoU* exists to allow temporal control of the pho regulon.

Table 6. Relative extracellular alkaline phosphatase activity of the indicated cultures

JCC#		5048	5048	5473	5473	6082	6082	6082	6082	6082	6082
PO4 State		Replete	Deplete	Deplete	Replete	Deplete	Replete	Replete	Replete	Deplete	Deplete
DOC (μM)		-	-	-	-	-	-	100	200	100	200
AP activity	24 (hr)	1.0	4.4	72.1	61.4	69.9	58.6	16.4	18.3	71.0	44.7
	48 (hr)	1.0	51.7	163.3	147.5	163.0	112.0	15.0	18.2	138.9	122.4

Example 7: Increased ethanol productivity with induced expression of phoU after phosphate depletion in the culture medium.

[00169] Culture conditions and sampling:

[00170] JCC5048 was grown in JB3.0, and JCC6082 was grown in JB3.0 with 0.6x phosphate with 10mM acetate. Cells were cultured in an indoor photobioreactor mimicking outdoor insolation with a peak insolation of 2000 uE/m2/s. At the time indicated by the arrow below, 20 µM cumate was added to the reactors.

[00171] Results

[00172] Induction of *phoU* following phosphate depletion in the medium allows increased ethanol productivity under these growth conditions as shown by the total ethanol produced (figure 21) and the daily ethanol productivities (figure 22).

Example 8: Preventing contaminant catabolism of extracellular products in fermentations of *E. coli* engineered to rapidly deplete the medium of phosphate

[00173] In order to improve microbial contaminant control in large-scale *E. coli* fermentations, strains of *E. coli* can be engineered to rapidly deplete the medium of phosphate similar to the previous examples given for *Synechococcus* sp. PCC7002. It has previously been shown that a *phoU* knockout of *E. coli* uptakes phosphate at a much higher rate as well and accumulates much more polyphosphate than the respective wild type strains (Morohoshi *et. al.* 2002). Inducible or modified constitutive regulation of phoU may also increase phosphate uptake and strain stability. Overexpression of polyphosphate kinase (*ppk*), acetate kinase (*ackA*) and/or phosphate transporter systems (*pst* operon) has also been carried out to increase phosphate uptake in *E. coli* (Kato *et. al.* 1993, Lee *et. al.* 2006). Over-expression of the PST transporter, either alone or in combination with PPK, did lead to a 3x increase in phosphate uptake over the wild type strain without loss of phosphate when the cultures stopped growing (Kato *et. al.* 1993).

[00174] As a proof of concept, *E. coli* strains co-expressing *pdc* and *adh* and showing improved phosphate uptake via some or all the genetic manipulations detailed above and also in Table 7 is evaluated for their ability to prevent contaminant catabolism of ethanol under fermentation conditions when three ethanol consuming heterotrophs are present (Table 3).

Phosphate-uptake regulation

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
phoU	*Escherichia coli*	b3724	NP_418180.1	phosphate transport regulatory protein	Modify expression

Polyphosphate formation

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
ppk	*Escherichia coli*	b2501	NP_416996.1	polyphosphate kinase	overexpress

Phosphate transport

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
pstS	*Escherichia coli*	b3728	NP_418184.1	periplasmic phosphate binding protein	overexpress
pstC		b3727	NP_418183.1	phosphate transporter subunit	
pstA		b3726	NP_418182.1	phosphate transporter subunit	
pstB		b3725	NP_418181.1	ATP-binding component, phosphate transporter subunit	

Phosphate metabolism

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
ackA	*Escherichia coli*	b2296	NP_416799.1	acetate kinase	overexpress

Phosphate-uptake regulation

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
phoU	*Escherichia coli*	b3724	NP_418180.1	phosphate transport regulatory protein	Modify expression

Polyphosphate formation

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
ppk	*Escherichia coli*	b2501	NP_416996.1	polyphosphate kinase	over-express

Phosphate transport

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
pstS	*Escherichia coli*	b3728	NP_418184.1	periplasmic phosphate binding protein	over-express
pstC		b3727	NP_418183.1	phosphate transporter subunit	
pstA		b3726	NP_418182.1	phosphate transporter subunit	
pstB		b3725	NP_418181.1	ATP-binding component, phosphate transporter subunit	

Phosphate metabolism

Gene ID	Organism	Locus ID	NCBI sequence	Function/annotation	Action
ackA	*Escherichia coli*	b2296	NP_416799.1	acetate kinase	over-express

Table 7. Representative *E. coli* genes and respective manipulations to increase phosphate uptake from medium.

Example 9: Preventing contaminant catabolism of extracellular products in fermentations of *Saccharomyces cerevisiae* engineered to rapidly deplete the medium of phosphate

[00175] In order to improve microbial contaminant control in large-scale yeast fermentations, strains of yeast like *Saccharomyces cerevisiae* can also be engineered to rapidly deplete the medium of phosphate. They store phosphate in the cell as polyphosphate similar to prokaryotes and also are capable of rapid uptake of phosphate. The phosphate uptake regulatory system is more complex (Ljundahl and Daignan-Fornier 2012), with 22 ORFs being involved in the rapid phosphate uptake response (Ogawa *et. al.* 2000). The transcription factor Pho4 plays an important role in the transcriptional regulation of the phosphate uptake response including control of high affinity phosphate transporters and polyphosphate biosynthetic genes. When wild-type *S. cerevisiae* is grown in phosphate replete conditions, Pho4 is phosphorylated by cyclin/kinase pair Pho80/Pho85 causing Pho4 to be exported from the nuclease to the cytoplasm. This prevents Pho4 from complexing with the transcriptional activator Pho2 and catalyzing increased expression of the phosphate uptake pathways. Knocking out the native Pho4 and expressing a mutant form named $Pho4^{SA1234PA6}$ that has four phosphorylation sites removed through serine -> alanine swaps and one site with a proline -> alanine swap has been shown to allow full induction of the phosphate-uptake genes in phosphate-replete media. Alternatively, expression of the native Pho4 in *S. cerevisiae* in a Δpho4 Δpho80 also allows high expression of the phosphate pathway when grown in replete phosphate conditions (Komeili and O'Shea 1999). Regulating Pho4, $Pho4^{SA1234PA6}$ or Pho80 expression under inducible promoters or other alternate promoters may allow optimal regulation of the pathway. Ensuring strong expression of the phosphate uptake pathway genes may not be enough however to deplete the phosphate in the medium, as post-translational regulation of phosphate uptake is known to exist is *S. cerevisiae*. For example, the primary high-affinity phosphate transporter under acidic conditions is Pho84, which appears to be removed from the membrane under high phosphate conditions even if the Pho84 gene is being strongly transcribed (Pratt *et. al.* 2004).

Therefore, mutants may need to be identified where the existing post-transcriptional mechanisms of phosphate control in *S. cerevisiae* are knocked out in order to allow high rates of phosphate uptake when phosphate is replete in the medium in addition to having the necessary transcriptional control of phosphate-uptake associated genes. It may also be necessary to express alternate/additional phosphate uptake transporters from different organisms or over-express native phosphate transporters to ensure optimal phosphate uptake kinetics.

[00176] As a proof of concept, *S. cerevisiae* strains co-expressing *pdc* and *adh* and showing improved phosphate uptake via the genetic manipulations detailed above is evaluated for their ability to prevent contaminant catabolism of ethanol under fermentation conditions when relevant microbial contaminants such as *Lactobacilli* and wild yeast (Beckner *et. al.* 2011) are present.

[00177] Citations

[00178] Beckner, M., Ivery, M.L. and Phister, T.G. 2011. Microbial contamination of fuel ethanol fermentations. Letters in Applied Microbiology. 53: 387-394.

[00179] Kato, J., Yamada, K., Muramatsu, A., Hardoyo and Ohtake, H. 1993. Genetic improvement of *Escherichia coli* for enhanced biological removal of phosphate from wastewater. Applied and Environmental Microbiology. 59: 3744-3749.

[00180] Komeili, A. and O'Shea, E.K. 1999. Roles of phosphorylation sites in regulating activity of the transcription factor Pho4. Science. 284: 977-980.

[00181] Lee, S.-J., Lee, Y.-S., Lee, Y.-C. and Choi, Y.-L. 2006. Molecular characterization of polyphosphate (polyP) operon from *Serratia marcescens*. Journal of Basic Microbiology. 46: 108-115.

[00182] Ljungdahl, P.O. and Baignan-Fornier. 2012. Regulation of amino acid, nucleotide, and phosphate metabolism in *Saccharomyces cerevisiae*. Genetics. 190:885-929.

[00183] Moroshi, T., Maruo, T., Shirai, Y., Kato, J., Ikeda, T., Takiguchi, N., Ohtake, H. and Kuroda, A. 2002. Accumulation of inorganic polyphosphate in phoU mutants of *Escherichia coli* and *Synechocystis* sp. strain PCC6803. Applied and Environmental Microbiology. 68: 4107-4110.

[00184] Ogawa, N., DeRisis, J. and Brown, P.O. 2000. New components of a system for phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae* revealed by genomic expression analysis. Molecular Biology of the Cell. 11: 4309-4321.

[00185] Pratt, J.R., Mouillon, J.-M., Lagerstadt, J.O., Pattison-Grandberg, J., Lundh, K.I. and Persson, B.L. 2004. Effects of methylphosphonate, a phosphate analogue, on the

expression and degradation of the high-affinity phosphate transporter Pho84, in *Saccharomyces cerevisiae*. Biochemistry. 43: 14444-14453.

[00186] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[00187] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

TABLE A

SEQ ID NO	DESCRIPTION	SEQUENCE
1	DNA sequence of pJB3730; 1st lowercase sequence: Upstream homology region for SYNPCC7002_A1708; 1st underlined sequence: *ermC* coding sequence; erythromycin selection marker; 2nd lowercase sequence: Downstream homology region for SYNPCC7002_A1708	TAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATAT TTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGA TCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCA AAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGT TTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCA TCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGGCGAAATACGCGATCGCTGTTAAAA GGACAATTACAAACAGGAATCGAGTGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTT TCACCTGAATCAGGATATTCTTCTAATACCTGGAACGCTGTTTTTCCGGGGATCGCAGTGGTGAGT AACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGTGGCATAAATTCCGTCAGC CAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAAC AACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGCCCGACATTATCG CGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCC CGTTGAATATGGCTCATATTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTC ATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTCAGTGTTACAACCAAT TAACCAATTCTGAACATTATCGCGAGCCCATTTATACCTGAATATGGCTCATAACACCCCTTGTTT GCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAG CGCCGATGGTAGTGTGGGGACTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAA AGGCTCAGTCGAAAGACTGGGCCTTTCGCCCGGGCTAATTAGGGGGTGTCGCCCTTTACACGTACT TAGTCGCTGAAGGCCTCACTGGCCCCTGCAGGgtcactaccccgaaaaaatccttatttttaccaa cgacaacgccaccgtttatcgcatctcccaggacttttaattcccgccattacccaccagacccc agtcaaagaacgccatgaaatcctcgaaaaatttcgtcgcgaaacataccatatcctggtgacttc ccatgttttaaatgaggggttgatgttcccaatgctagagtggcaattattctctccggcagcgg ttcgagtcgggaatatgtacaacgtttagggcgaattttacgcaaaggtaaatcagcgacaaaatt ggctcttttgtatgaattggtagcagaaaatacaaacgaagagcaactttcccaacggcgacgttc tcccctaacccaacaaagcaaccgcttacctctataaattcaaacagtaatttaccacgggttgc agaagcccaaagcccttataaagcaaacttcccagagaaatctgaacagcccaaagacccacccat ttgaTTAATTAAGAGCATCTCTTCGAAGTATTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAA AGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTC ACCTTCGGGTGGGCCTTTCTGCGTTTATACGGTGCACTTTAGGTGAATAAGTTGTATATTTAAAAT CTCTTTAATTATCAGTAAATTAATGTAAGTAGGTCATTATTAGTCAAAATAAAATCATTTGTCGAT TTCAATTTTGGCATGACTTGAGCGCATAGCCAAGGGGTGTTATGAGCCATATTCAGGTATAAATGG GCTCGCGATAATGTTCAGAATTGGTTAATTGGTTGTAACACTGACCCCTATTTGTTTATTTTTCTA AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAA AAGGAGACAATTGATGAACGAGAAAAATATAAAACACAGTCAAAACTTTATTACTTCAAAACATAA

		TATAGATAAAATAATGACAAATATAAGATTAAATGAACATGATAATATCTTTGAAATCGGCTCAGG AAAAGGCCATTTTACCCTTGAATTAGTAAAGAGGTGTAATTTCGTAACTGCCATTGAAATAGACCA TAAATTATGCAAAACTACAGAAAATAAACTTGTTGATCACGATAATTTCCAAGTTTTAAACAAGGA TATATTGCAGTTTAAATTTCCTAAAAACCAATCCTATAAAATATATGGTAATATACCTTATAACAT AAGTACGGATATAATACGCAAAATTGTTTTTGATAGTATAGCTAATGAGATTTATTTAATCGTGGA ATACGGGTTTGCTAAAAGATTATTAAATACAAAACGCTCATTGGCATTACTTTTAATGGCAGAAGT TGATATTTCTATATTAAGTATGGTTCCAAGAGAATATTTTCATCCTAAACCTAAAGTGAATAGCTC ACTTATCAGATTAAGTAGAAAAAAATCAAGAATATCACACAAAGATAAACAAAAGTATAATTATTT CGTTATGAAATGGGTTAACAAAGAATACAAGAAAATATTTACAAAAAATCAATTTAACAATTCCTT AAAACATGCAGGAATTGACGATTTAAACAATATTAGCTTTGAACAATTCTTATCTCTTTTCAATAG CTATAAATTATTTAATAAGTAAGCATGCACACTGAACAGTCAATGCTCTGTCTCAGGTCACTAATA CTATCTAAGTAGTTGATTCATAGTGACTGGATATGTTGCGTTTTGTCGCATTATGTAGTCTATCAT TTAACCACAGATTAGTGTAATGCGATGATTTTTAAGTGATTAATGTTATTTTGTCATCCTTTAGGT GAAAAAGGTTGAGTGCAACGTAAAAAAACCCGCCCCGGCGGGTTTTTTTATACCGGCGCGCCcacc cctctagctccctttttgagaaacatccacacccctctagctcccctgaggggagaagattggcc accttccccaaggcagaaccccttccggcctagcggccaccttccccaaggaaggatagtgggttt attgtgtggcgtcggagaggtcgaccacgtcaagccagccttcgagggcatcccagccgaggcctt tacggatgagggtcggttggcgatcgcaaaagtcgagaatcgttgaagttttggtgccgggctcgc tgtcactgtcgatgatgatatccacaaggggctctagggcatcaaagaggcgcgcttttttcaaggc caagggtaaaaggttcaccatcttcgtcgggcaagtgggccgaggtggaaataatcggattgccaa gggcctggagtaaaccctgacagatcgcatggtcagggacacgaatcccggtgctgcggcgtttgg ggctcatcaccagcttcggcacctgttttgtggcGGCCGGCCTACATGGCCCGTCAATCGAAGGGC GACACAAAATTTATTCTAAATGCATAATAAATACTGATAACATCTTATAGTTTGTATTATATTTTG TATTATCGTTGACATGTATAATTTTGATATCAAAAACTGATTTTCCCTTTATTATTTTCGAGATTT ATTTTCTTAATTCTCTTTAACAAACTAGAAATATTGTATATACAAAAAATCATAAATAATAGATGA ATAGTTTAATTATAGGTGTTCATCAATCGAAAAAGCAACGTATCTTATTTAAAGTGCGTTGCTTTT TTCTCATTTATAAGGTTAAATAATTCTCATATATCAAGCAAAGTGACAGGCGCCCTTAAATATTCT GACAAATGCTCTTTCCCTAAACTCCCCCCATAAAAAAACCCGCCGAAGCGGGTTTTTACGTTATTT GCGGATTAACGATTACTCGTTATCAGAACCGCCCAGGGGGCCCGAGCTTAAGACTGGCCGTCGTTT TACAACACAGAAAGAGTTTGTAGAAACGCAAAAAGGCCATCCGTCAGGGGCCTTCTGCTTAGTTTG ATGCCTGGCAGTTCCCTACTCTCGCCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTT CGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGAT AACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTG CTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG TGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTT TCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTG CACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCG GTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA GGCGGTGCTACAGAGTTCTTGAAGTGGTGGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGT ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAA ACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCT CAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGACGCGCGCGTAACTCAC GTTAAGGGATTTTGGTCATGAGCTTGCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCTTT
2	P(cum02) promoter	gcacatgtctagcgcttgaatttcgcgtaccgctctcgcgcaatttcgagtgtcgagttcctgaca cgctcaaagcgttctttgtccttctgccataggctacgaacagcaagcccacgcaccgaattgaat atcaaccaaaggatatcttctgcatcatcacgcgaaagaccacggctcaccagaacaccaagccac atatcctcgacgacaaaccgattcctctctaccgtgcgctgaataccctcgcgtaacgctggatcc cggtcggcagccacaatcaaatcaaggctgatagagaagtcatcgtcgaggaaaaattcggcggcg tcgtccagcatttgctggatgacgtcatcctctggcttcaatttcgctaatcgagcccgactgcgt tcggtgatctgttcgtaaagccattcaaaagtggcaagcagaagctcaagctttgtcgggaaatga

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4	DNA sequence of pJB4718; 1st lowercase sequence: Upstream homology region for SYNPCC7002_A1708; 1st uppercase sequence: P (BreO2.1_theoRS) promoter; 2no lowercase sequence: SYNPCC7002_A1708 coding sequence; 1st underlined sequence: *bla* resistance marker; 2nd underlined sequence: Downstream homology region for SYNPCC7002_A1708: 3rd underlined sequence:	ggccggcctacatggcccgtcaatcgaagggcgacacaaaatttattctaaatgcataataaatac tgataacatcttatagtttgtattatattttgtattatcgttgacatgtataattttgatatcaaa aactgatttttcccttttattattttcgagatttatttttcttaattctctttaacaaactagaaatat tgtatatacaaaaaatcataaataatagatgaatagtttaattataggtgttcatcaatcgaaaaa gcaacgtatcttatttaaagtgcgttgctttttttctcatttataaggttaaataattctcatatat caagcaaagtgacaggcgcccttaaatattctgacaaatgctctttccctaaactcccccatataa aaaacccgccgaagcgggtttttacgttatttgcggattaacgattactcgttatcagaaccgccc aggggcccgagcttaagactggccgtcgttttacaacacagaaagagtttgtagaaacgcaaaaa ggccatccgtcaggggccttctgcttagtttgatgcctggcagttccctactctcgccttccgctt cctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaagg cggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagc aaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccctgacg agcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccagg cgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgt ccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcgg tgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgcct tatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagcca ctggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtgggcta actacggctacactagaagaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaa aaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgca agcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctg acgctcagtggaacgacgcgcgcgtaactcacgttaagggattttggtcatgagcttgcgccgtcc cgtcaagtcagcgtaatgctctgcttttagaaaaactcatcgagcatcaaatgaaactgcaatttta ttcatatcaggattatcaataccatatttttgaaaaagccgtttctgtaatgaaggagaaaactca ccgaggcagttccataggatggcaagatcctggtatcggtctgcgattccgactcgtccaacatca atacaacctattaatttcccctcgtcaaaaataaggttatcaagtgagaaatcaccatgagtgacg actgaatccggtgagaatggcaaaagtttatgcatttctttccagacttgttcaacaggccagcca ttacgctcgtcatcaaaatcactcgcatcaaccaaaccgttattcattcgtgattgcgcctgagcg aggcgaaatacgcgatcgctgttaaaaggacaattacaaacaggaatcgagtgcaaccggcgcagg aacactgccagcgcatcaacaatattttcacctgaatcaggatattcttctaatacctggaacgct gtttttccggggatcgcagtggtgagtaaccatgcatcatcaggagtacggataaaatgcttgatg gtcggaagtggcataaattccgtcagccagtttagtctgaccatctcatctgtaacatcattggca

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What is claimed is:

1. An engineered photosynthetic microorganism modified to increase internal storage of one or more nutrients relative to an otherwise identical control microorganism that is not modified to increase internal storage of the one or more nutrients, optionally wherein the one or more nutrients comprises phosphorus, sulfur, nitrogen, carbon, iron, manganese, cobalt, zinc, molybdenum, copper, boron, or combinations thereof, optionally wherein the engineered photosynthetic microorganism comprises one or more recombinant nucleotide sequences encoding one or more proteins capable of producing one or more carbon-based product(s) of interest, and optionally wherein the modification to increase internal storage of one or more nutrients is one or more modifications to the expression or regulation of *phoU* gene(s) or one or more modifications to the expression or regulation of phoU protein(s) of the microorganism, wherein the expression or activity of the protein encoded by the modified *phoU* gene(s) or the expression or activity of the phoU protein(s) can be or is reduced relative to an otherwise identical protein in an otherwise identical microorganism lacking the one or more modifications.

2. The engineered microorganism of claim 1, wherein the expression or activity of the protein encoded by the *phoU* gene(s) is eliminated and/or knocked out.

3. The engineered microorganism of claim 1, wherein the one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest comprise *adh* and/or *pdc*.

4. The engineered microorganism of claim 1, wherein an inducible or repressible promoter operably linked to the coding sequence of the *phoU* gene(s).

5. The engineered microorganism of claim 4, wherein the expression profile of the *phoU* gene(s) can be altered via the promoter relative to the otherwise identical microorganism lacking said one or more modifications.

6. The engineered microorganism of claim 4, wherein the expression profile of the *phoU* gene(s) can be temporally altered via the promoter relative to the otherwise identical microorganism lacking said one or more modifications.

7. The engineered microorganism of claim 1, wherein the expression profile of the *phoU* gene(s) can be altered via one or more modifications.

8. The engineered microorganism of claim 1, wherein the one or more modifications comprise one or more mutations in the modified *phoU* gene(s).

9. The engineered microorganism of claim 8, wherein the modified *phoU* gene is a deleted *phoU* gene(s).

10. The engineered microorganism of claim 1, wherein the one or modifications comprise cleavage or silencing of the mRNA product(s) of the *phoU* gene(s) via RNA interference (RNAi).

11. The engineered microorganism of claim 1, wherein the microorganism further comprises one or more recombinant nucleotide sequences encoding a catalase or wherein the microorganism further comprises a catalase.

12. The engineered microorganism of claim 1, wherein the carbon-based product(s) of interest comprises or is ethanol or an alkane.

13. The engineered microorganism of claim 1, wherein the phosphate uptake rate of the microorganism is 2, 3, 4, 5, 6, 7, 8, or 9 times the rate of the phosphate uptake in an otherwise identical microorganism lacking said one or more modifications and cultured under identical conditions.

14. The engineered microorganism of claim 1, wherein the phosphate uptake rate of the microorganism is about 9 times the rate of the phosphate uptake in an otherwise identical microorganism lacking said one or more modifications and cultured under identical conditions.

15. The engineered microorganism of claim 1, wherein the phosphate uptake rate of the microorganism is greater than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300 $\mu M\ h^{-1}$.

16. The engineered microorganism of claim 1, wherein the phosphate uptake rate of the microorganism is about 80 to 300 $\mu M\ h^{-1}$.

17. The engineered microorganism of claim 1, wherein the microorganism is capable of producing an equivalent rate and/or amount of carbon-based product(s) of interest in both the presence and absence of a distinct microorganism.

18. The engineered microorganism of claim 17, wherein distinct microorganism is a carbon-based product(s) of interest catabolizing microorganism.

19. The engineered microorganism of claim 17, wherein the distinct microorganism comprises *Acinetobacter baylyi* ADP1, *Bacillus thuringiensis*, and/or *Pseudomonas stutzeri*.

20. The engineered microorganism of claim 17, wherein the distinct microorganism is present at less than 10, 10 or greater than 10 colony forming units (CFU)/ml.

21. The engineered microorganism of claim 17, wherein the distinct microorganism is present at 10^3 CFU/ml or less.

22. The engineered microorganism of claim 1, wherein the microorganism is capable of producing carbon-based product(s) of interest over an increased period of time in the presence of acetate.

23. The engineered microorganism of claim 1, wherein the microorganism is capable of producing carbon-based product(s) of interest over an increased period of time in the presence of 10 millimolar acetate.

24. The engineered microorganism of claim 1, wherein said one or more recombinant genes is integrated into the genome of said microorganism.

25. The engineered microorganism of claim 1, wherein said one or more recombinant genes is extrachromosomal.

26. The engineered microorganism of claim 1, wherein said microorganism is a cyanobacterium.

27. The engineered microorganism of claim 1, wherein said microorganism is a thermotolerant cyanobacterium.

28. The engineered microorganism of claim 1, wherein said microorganism is a *Synechococcus* species.

29. A cell culture comprising a culture medium and the microorganism of any one of claims 1-28.

30. The culture of claim 29, further comprising acetate.

31. The culture of claim 29, further comprising one or more distinct microorganisms.

32. The culture of claim 31, wherein the one or more distinct microorganisms are carbon-based product(s) of interest catabolizing microorganisms.

33. The culture of claim 31, wherein the distinct microorganism is present at less than 10, 10, or greater than 10 CFU/ml.

34. The culture of claim 31, wherein the distinct microorganism is present at 10^3 CFU/ml or less.

35. The culture of claim 31, wherein contaminant growth is slowed or stopped when phosphate is depleted from the medium.

36. A method for producing carbon-based product(s) of interest, comprising:

culturing the engineered photosynthetic microorganism of any of claims 1-28 in a culture medium, wherein said engineered microorganism produces equivalent or increased amounts of carbon-based product(s) of interest relative to an otherwise identical microorganism, cultured under identical conditions, but lacking said one or more modifications.

37. The method of claim 36, further comprising allowing the carbon-based product(s) of interest to accumulate in the culture.

38. The method of claim 36, further comprising isolating at least a portion of the carbon-based product(s) of interest from said culture.

39. The method of claim 38, further comprising processing the isolated carbon-based product(s) of interest to produce a processed compound.

40. A composition comprising carbon-based product(s) of interest, wherein said carbon-based product(s) of interest is produced by the method of any one of claims 36-39

41. The composition of claim 40, wherein the composition comprises at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% carbon-based product(s) of interest.

42. A method for producing carbon-based product(s) of interest, comprising:

(i) culturing an engineered photosynthetic microorganism of any of claims 1-28 in a culture medium; and

(ii) exposing said engineered photosynthetic microorganism to light and inorganic carbon, wherein said exposure results in the conversion of said inorganic carbon by said microorganism into carbon-based product(s) of interest in an amount greater than or equal to that converted by an otherwise identical photosynthetic microorganism, cultured under identical conditions, but lacking said one or more modifications.

43. The method of claim 42, further comprising allowing the carbon-based product(s) of interest to accumulate in the culture.

44. The method of claim 42, further comprising isolating at least a portion of the carbon-based product(s) of interest from said culture.

45. The method of claim 44, further comprising processing the isolated carbon-based product(s) of interest to produce a processed compound.

46. A composition comprising carbon-based product(s) of interest, wherein said carbon-based product(s) of interest is produced by the method of any one of claims 42-45.

47. The composition of claim 46, wherein the composition comprises at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% carbon-based product(s) of interest.

48. A method for controlling contaminants comprising:

culturing a photosynthetic microorganism characterized in its capacity to uptake one or more nutrients in a culture medium; and

limiting the one or more nutrients in the culture medium to the contaminants.

49. The method of claim 48 wherein the one or more nutrients comprise phosphorus, sulfur, nitrogen, carbon, iron, manganese, cobalt, zinc, molybdenum, copper, and/or boron.

50. The method of claim 48 wherein the photosynthetic microorganism stores the one or more nutrients.

51. The method of claim 48 wherein the photosynthetic microorganism is not limited for the one or more nutrients in the culture medium.

52. The method of claim 48 wherein the photosynthetic microorganism comprises one or more genetic modifications or is cultured under conditions to deplete the nutrients from the culture medium.

53. The method of claim 48 wherein the contaminants comprise one or more heterotrophs.

54. The method of claim 53 wherein the heterotrophs are selected from *Acinetobacter* sp., *Bacillus* sp., or *Pseudomonas* sp.

55. The method of claim 48 wherein the photosynthetic microorganism comprises one or more recombinant nucleotide sequences encoding one or more proteins capable of producing carbon-based product(s) of interest.

56. The method of claim 55 wherein the culturing step comprises exposing the engineered photosynthetic microorganism to light and inorganic carbon, wherein the exposure results in the conversion of the inorganic carbon by the photosynthetic microorganism into carbon-based product(s) of interest.

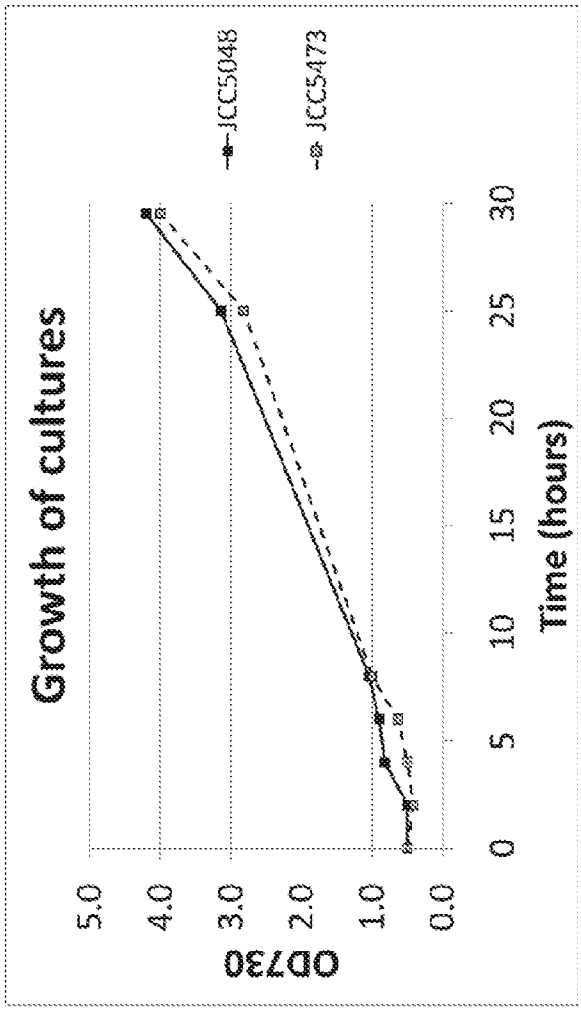
Growth of cultures
OD730
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
25
30
Time (hours)
JCC5048
JCC5473

Figure 1

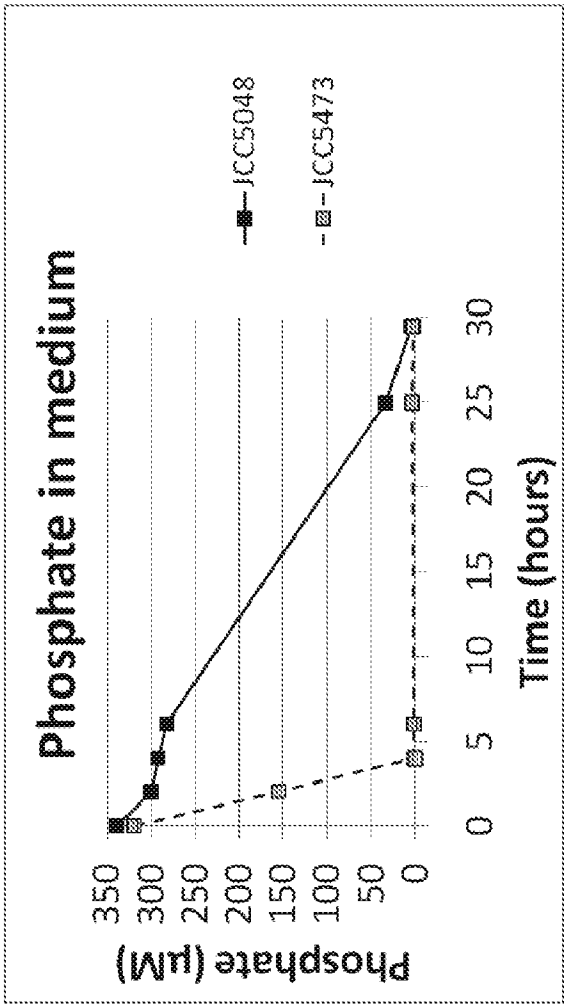
Phosphate in medium
JCC5048
JCC5473
Phosphate (µM)
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (hours)

Figure 2

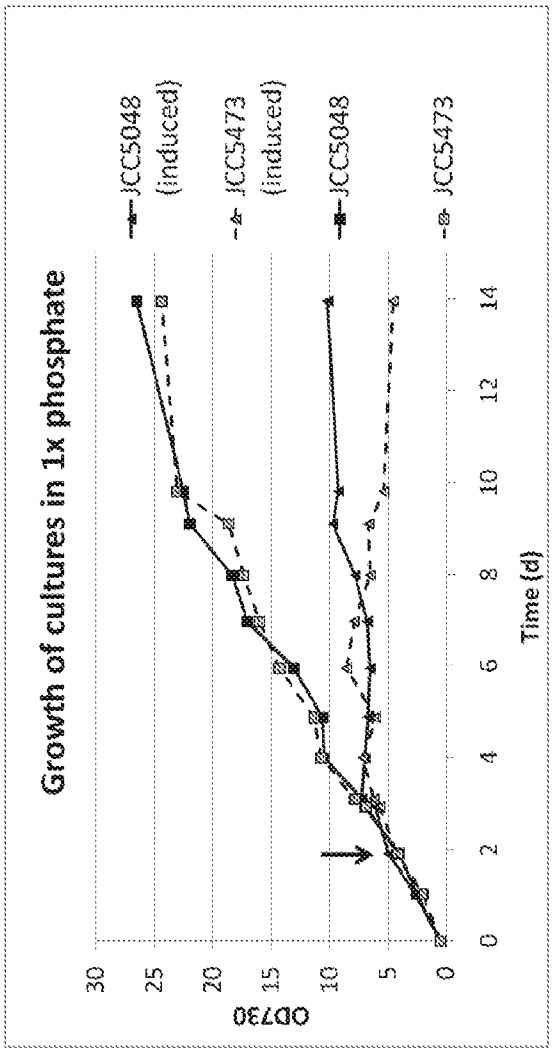
Growth of cultures in 1x phosphate
JCC5048 (induced)
JCC5473 (induced)
JCC5048
JCC5473
OD730
Time (d)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14

Figure 3

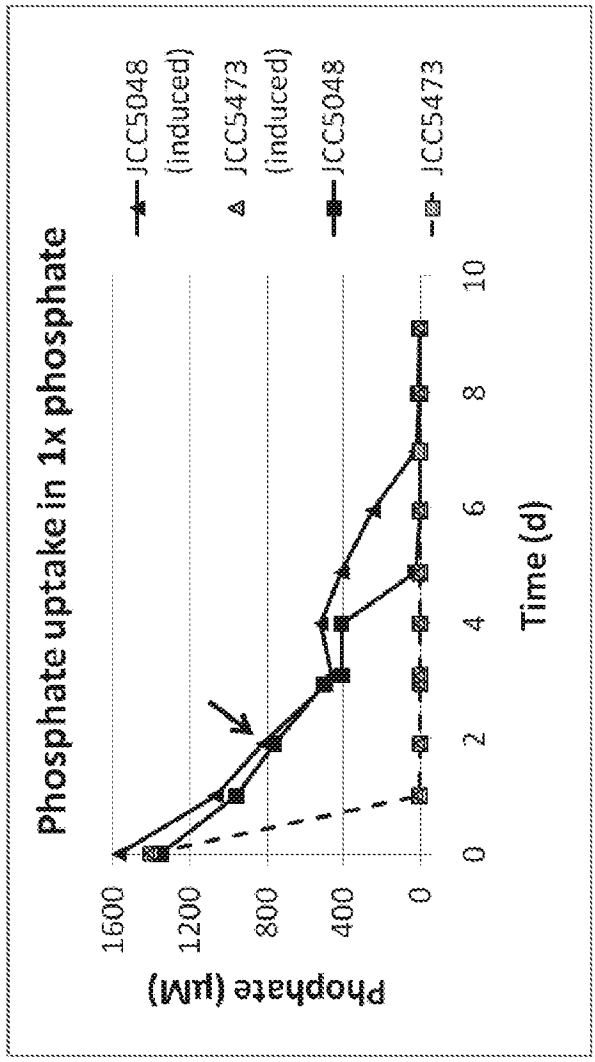
Phosphate uptake in 1x phosphate
JCC5048 (induced)
JCC5473 (induced)
JCC5048
JCC5473
Phophate (µM)
1600
1200
800
400
0
0
2
4
6
8
10
Time (d)

Figure 4

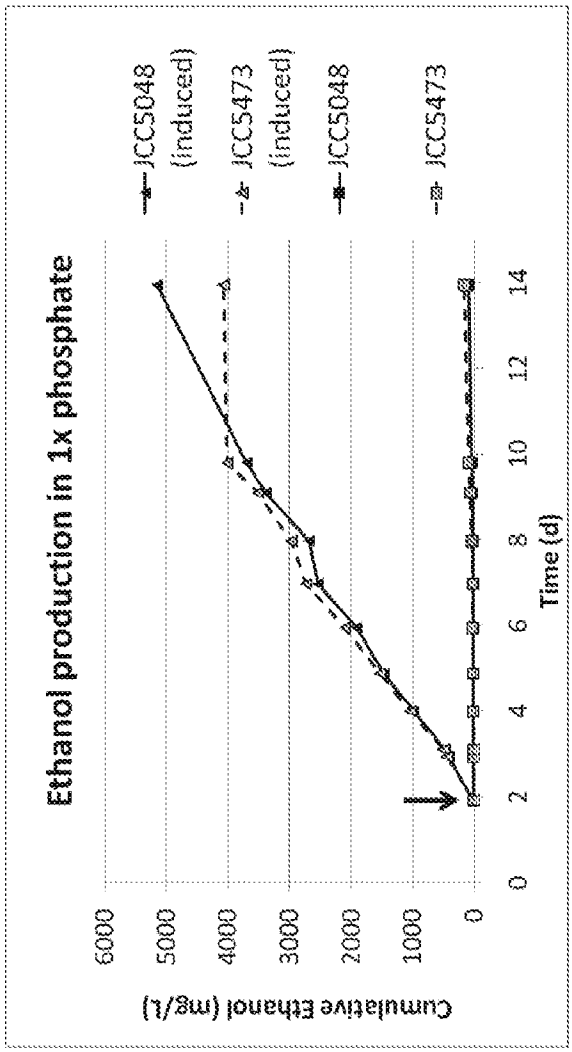
Ethanol production in 1x phosphate
JCC5048 (induced)
JCC5473 (induced)
JCC5048
JCC5473
Cumulative Ethanol (mg/L)
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
Time (d)

Figure 5

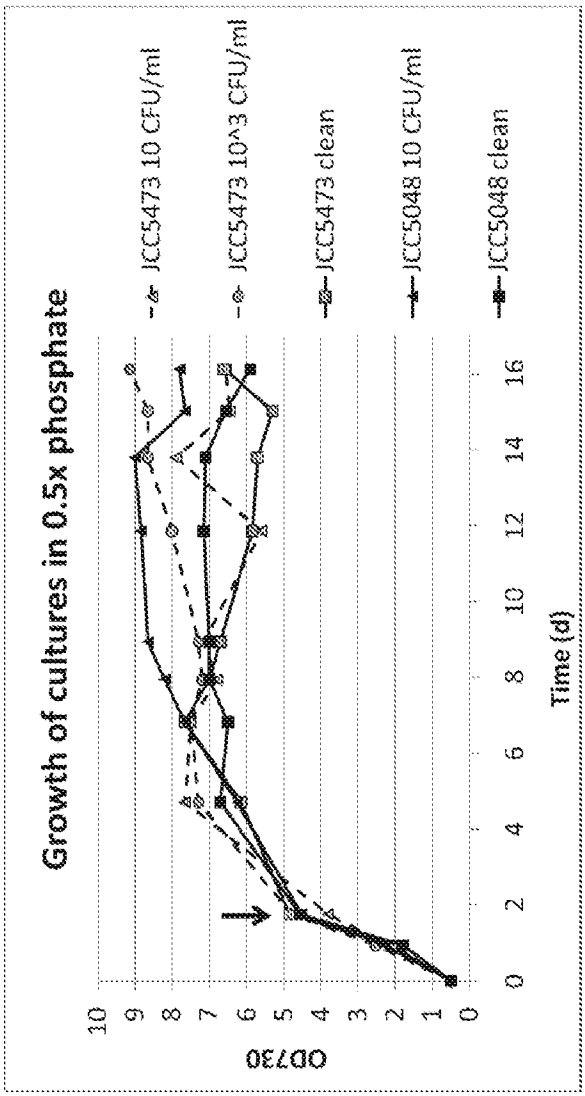
Growth of cultures in 0.5x phosphate
JCC5473 10 CFU/ml
JCC5473 10^3 CFU/ml
JCC5473 clean
JCC5048 10 CFU/ml
JCC5048 clean
OD730
Time (d)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16

Figure 6

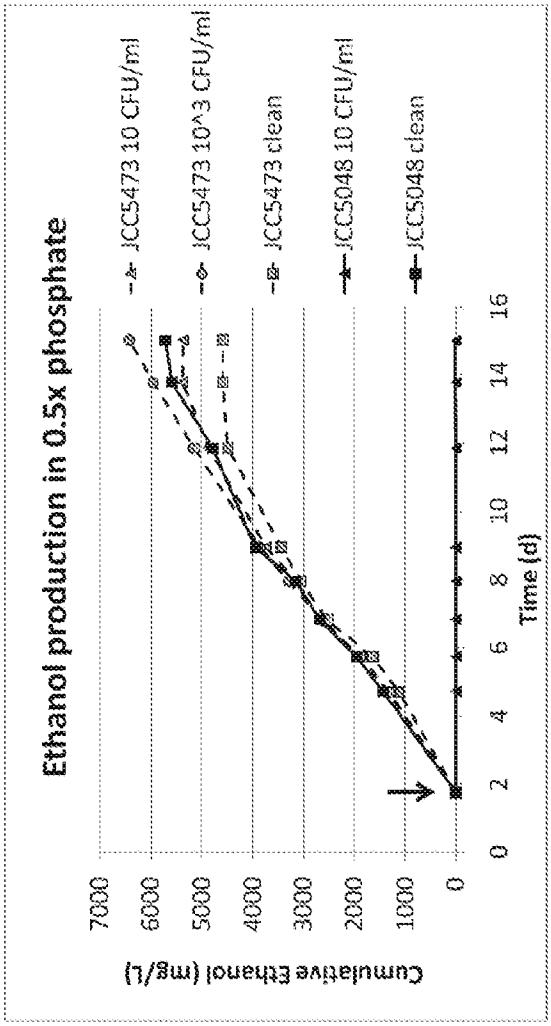
Ethanol production in 0.5x phosphate
JCC5473 10 CFU/ml
JCC5473 10^3 CFU/ml
JCC5473 clean
JCC5048 10 CFU/ml
JCC5048 clean
Cumulative Ethanol (mg/L)
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
16
Time (d)

Figure 7

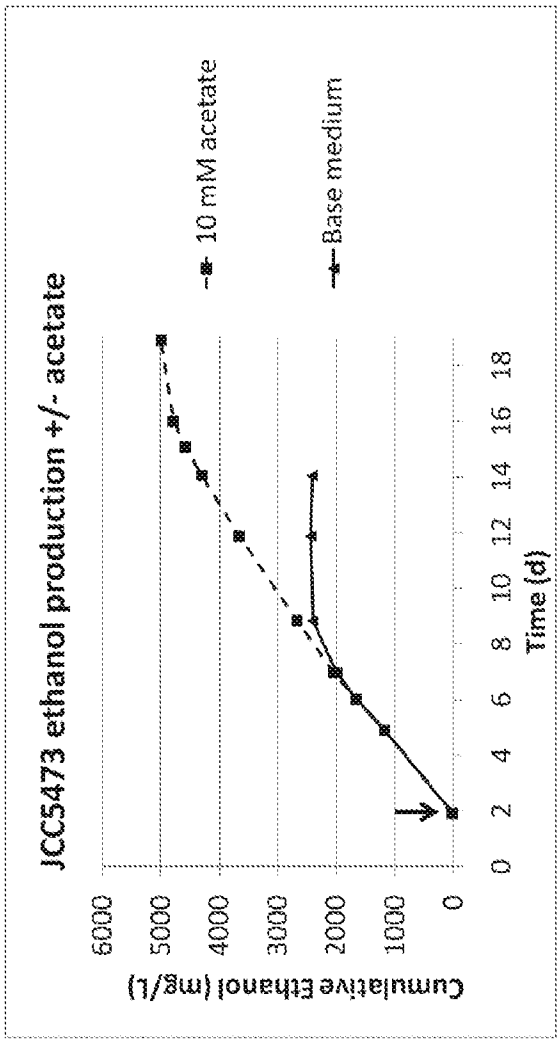
JCC5473 ethanol production +/- acetate
10 mM acetate
Base medium
Cumulative ethanol (mg/L)
6000
5000
4000
3000
2000
1000
0
0 2 4 6 8 10 12 14 16 18
Time (d)

Figure 8

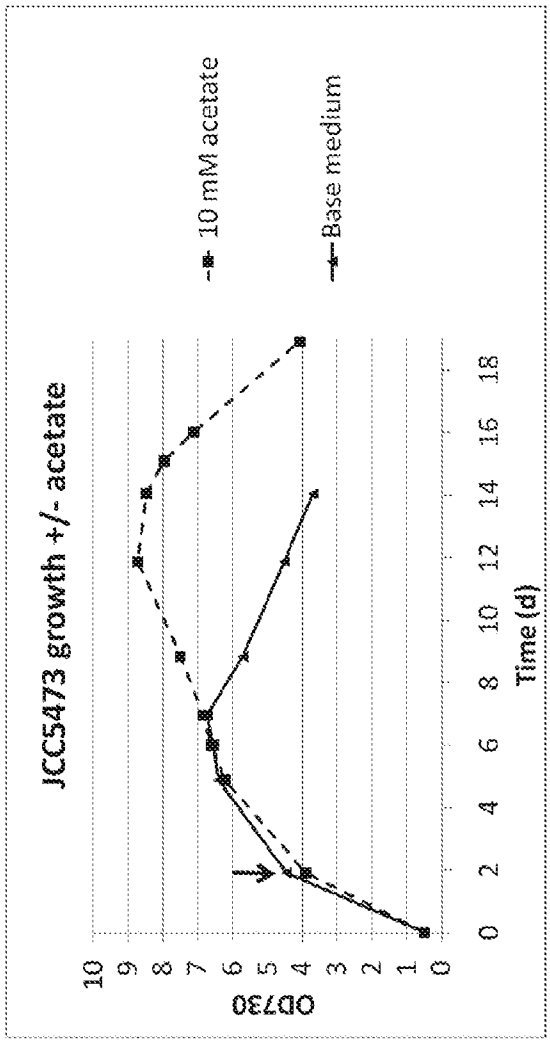
JCC5473 growth +/- acetate
10 mM acetate
Base medium
OD730
Time (d)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18

Figure 9

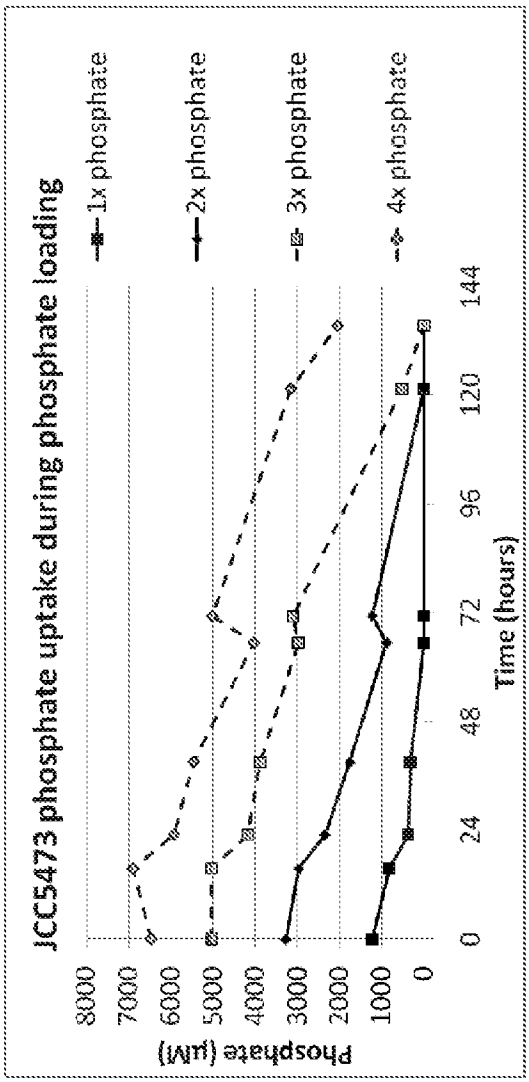
JCC5473 phosphate uptake during phosphate loading
1x phosphate
2x phosphate
3x phosphate
4x phosphate
Phosphate (µM)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
24
48
72
96
120
144
Time (hours)

Figure 10

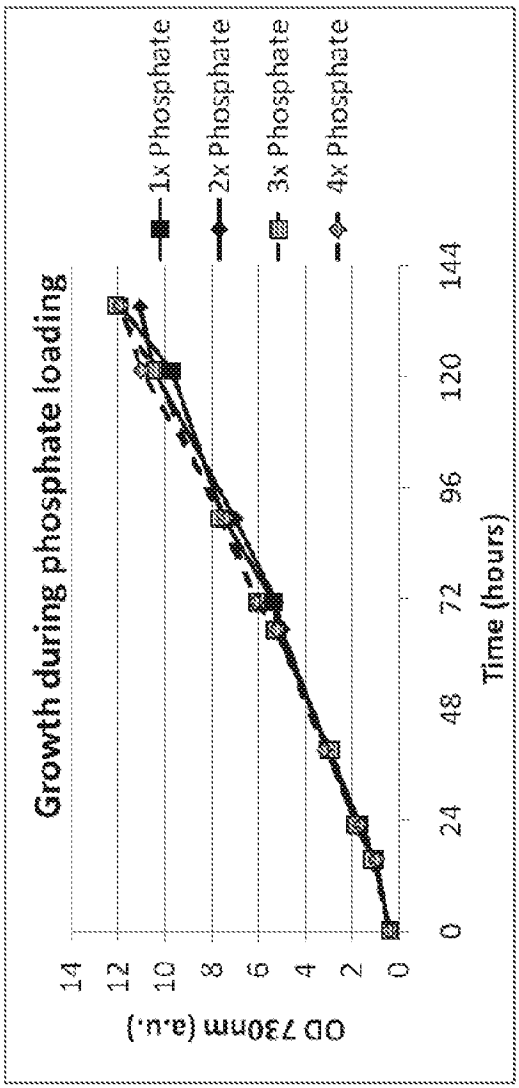
Growth during phosphate loading
OD 730nm (a.u.)
0
2
4
6
8
10
12
14
0
24
48
72
96
120
144
Time (hours)
1x Phosphate
2x Phosphate
3x Phosphate
4x Phosphate

Figure 11

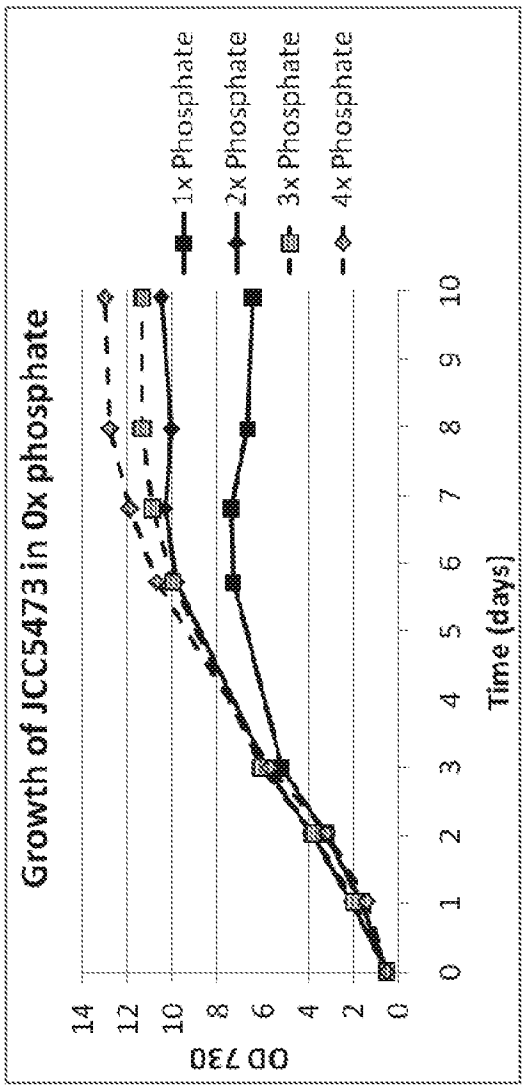
Growth of JCC5473 in 0x phosphate
OD 730
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10
Time (days)
1x Phosphate
2x Phosphate
3x Phosphate
4x Phosphate

Figure 12

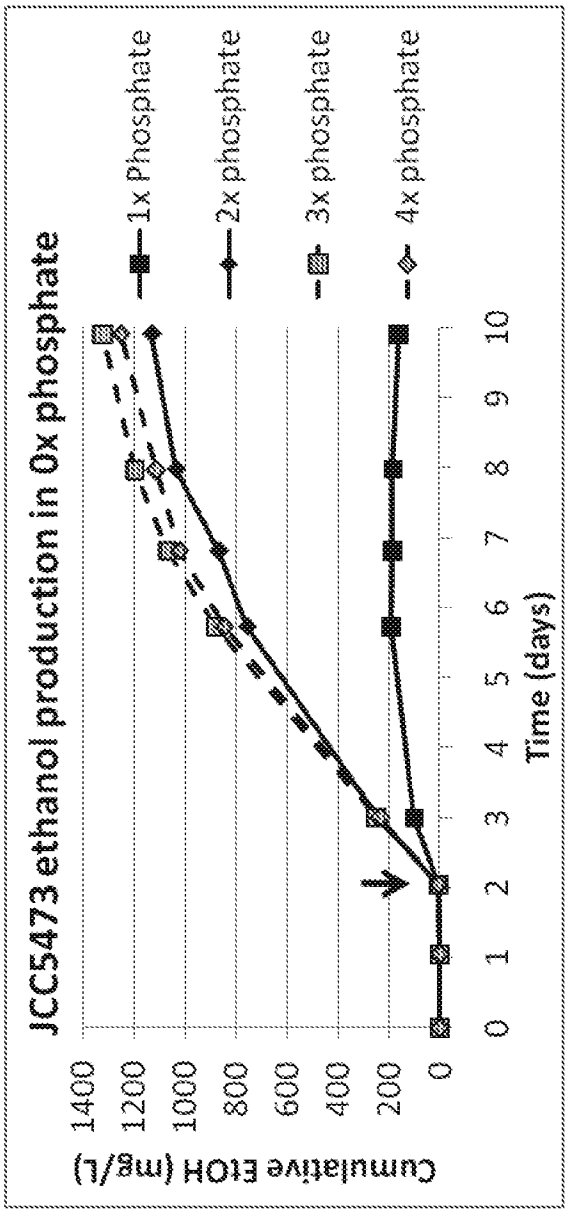
JCC5473 ethanol production in 0x phosphate
Cumulative EtOH (mg/L)
1400
1200
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10
Time (days)
1x Phosphate
2x phosphate
3x phosphate
4x phosphate

Figure 13

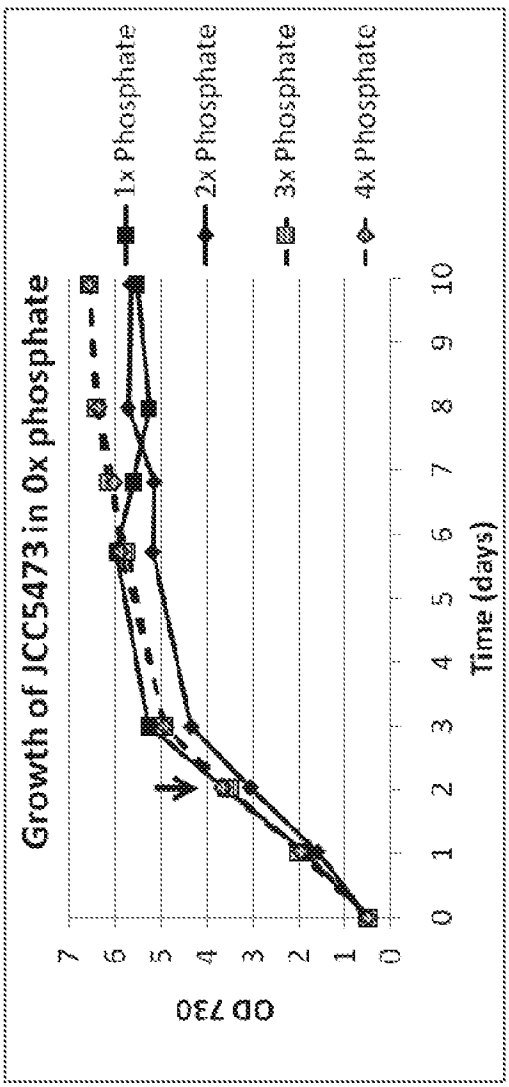
Growth of JCC5473 in 0x phosphate
1x Phosphate
2x Phosphate
3x Phosphate
4x Phosphate
OD 730
0
1
2
3
4
5
6
7
Time (days)
0
1
2
3
4
5
6
7
8
9
10

Figure 14

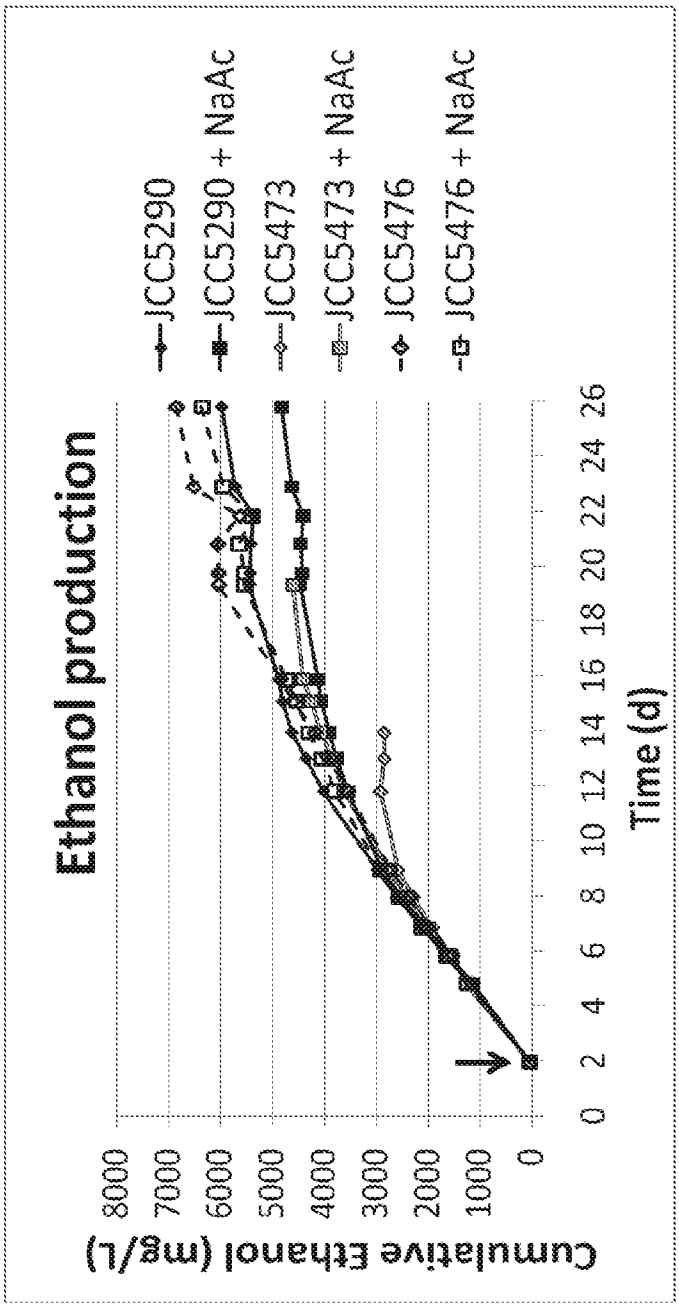
Ethanol production
JCC5290
JCC5290 + NaAc
JCC5473
JCC5473 + NaAc
JCC5476
JCC5476 + NaAc
Cumulative Ethanol (mg/L)
8000
7000
6000
5000
4000
3000
2000
1000
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Time (d)

Figure 15

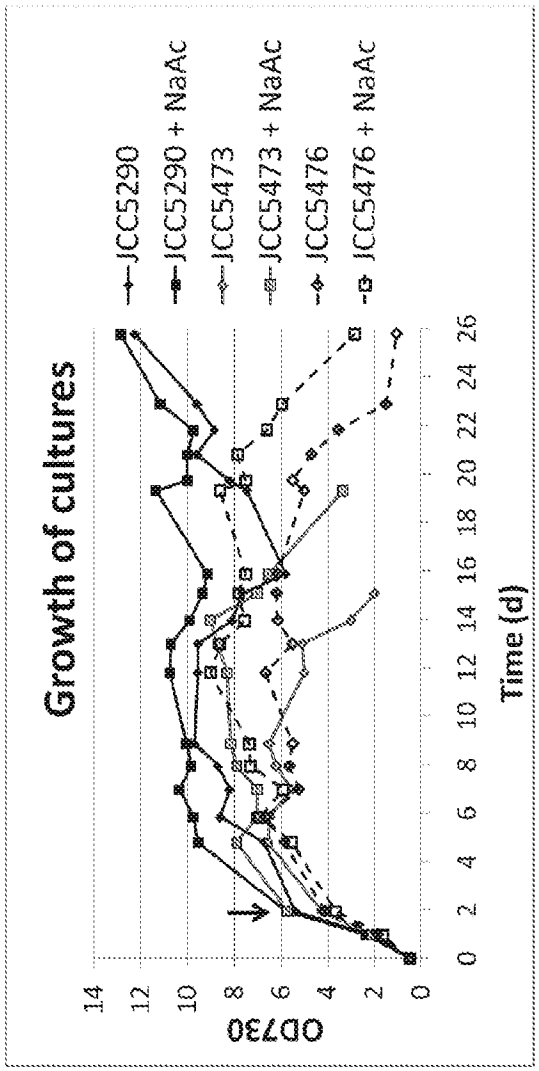
Growth of cultures
JCC5290
JCC5290 + NaAc
JCC5473
JCC5473 + NaAc
JCC5476
JCC5476 + NaAc
OD730
Time (d)
0
2
4
6
8
10
12
14
16
18
20
22
24
26

Figure 16

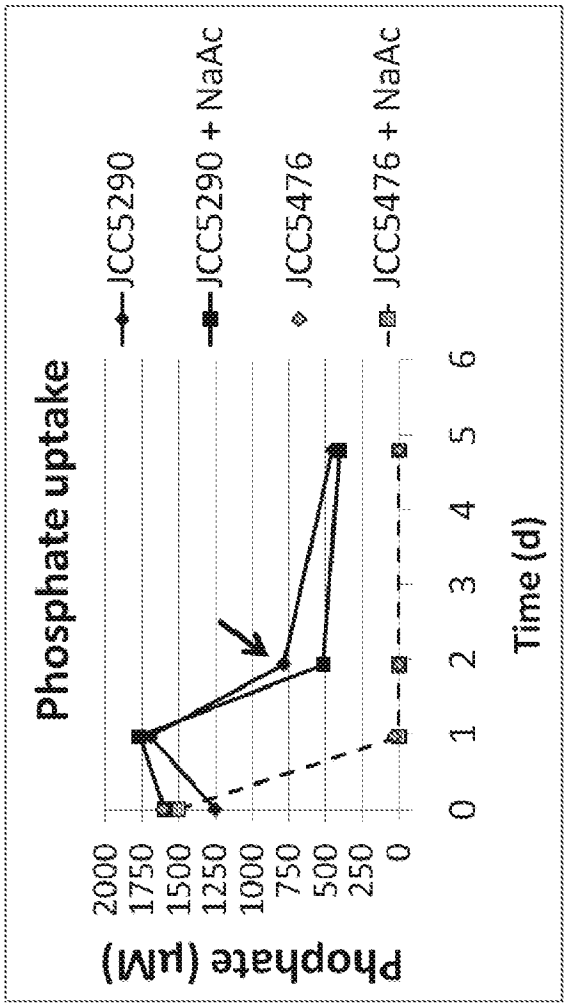
Phosphate uptake
JCC5290
JCC5290 + NaAc
JCC5476
JCC5476 + NaAc
Phophate (µM)
2000
1750
1500
1250
1000
750
500
250
0
0 1 2 3 4 5 6
Time (d)

Figure 17

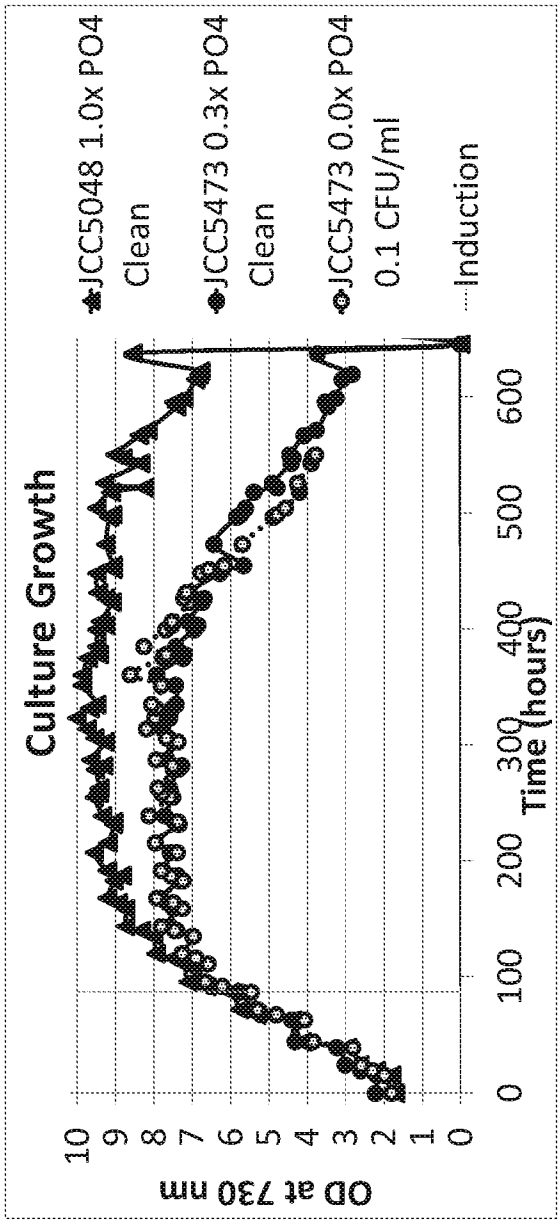
Culture Growth
JCC5048 1.0x PO4
Clean
JCC5473 0.3x PO4
Clean
JCC5473 0.0x PO4
0.1 CFU/ml
Induction
OD at 730 nm
Time (hours)
0
1
2
3
4
5
6
7
8
9
10
0
100
200
300
400
500
600

Figure 18

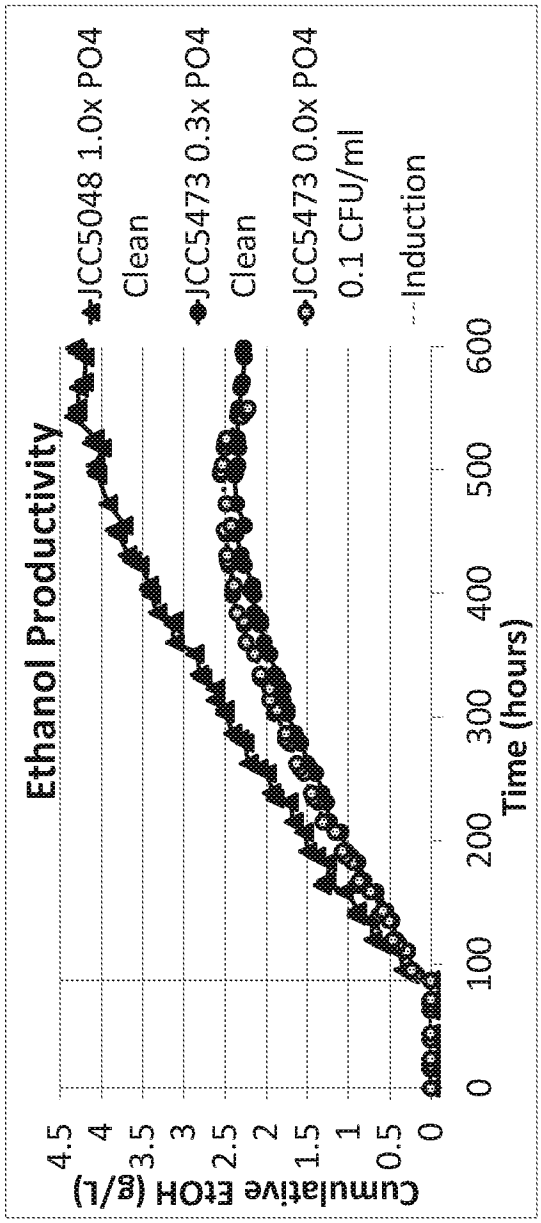
Ethanol Productivity
JCC5048 1.0x PO4
Clean
JCC5473 0.3x PO4
Clean
JCC5473 0.0x PO4
0.1 CFU/ml
Induction
Cumulative EtOH (g/L)
Time (hours)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
100
200
300
400
500
600

Figure 19

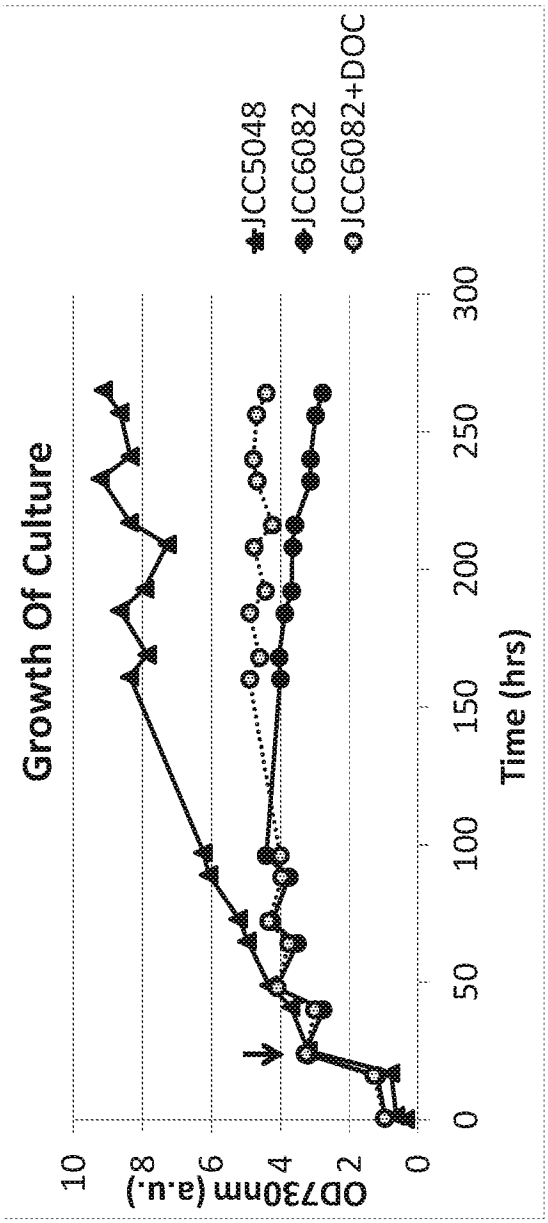
Growth Of Culture
OD730nm (a.u.)
Time (hrs)
JCC5048
JCC6082
JCC6082+DOC
0
2
4
6
8
10
0
50
100
150
200
250
300

Figure 20

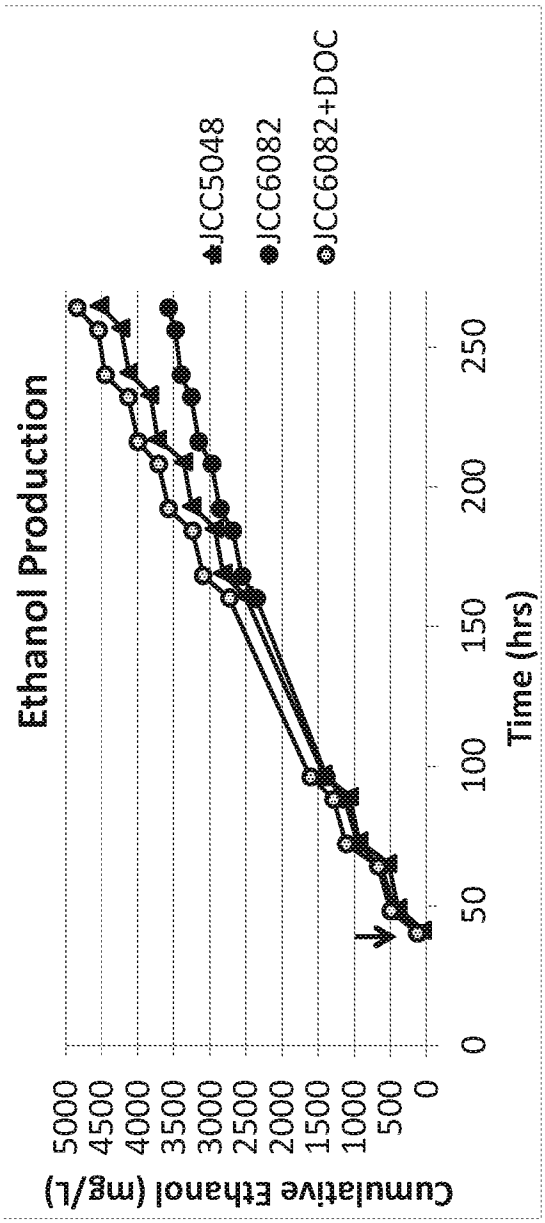
Ethanol Production
Cumulative Ethanol (mg/L)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
250
Time (hrs)
JCC5048
JCC6082
JCC6082+DOC

Figure 21

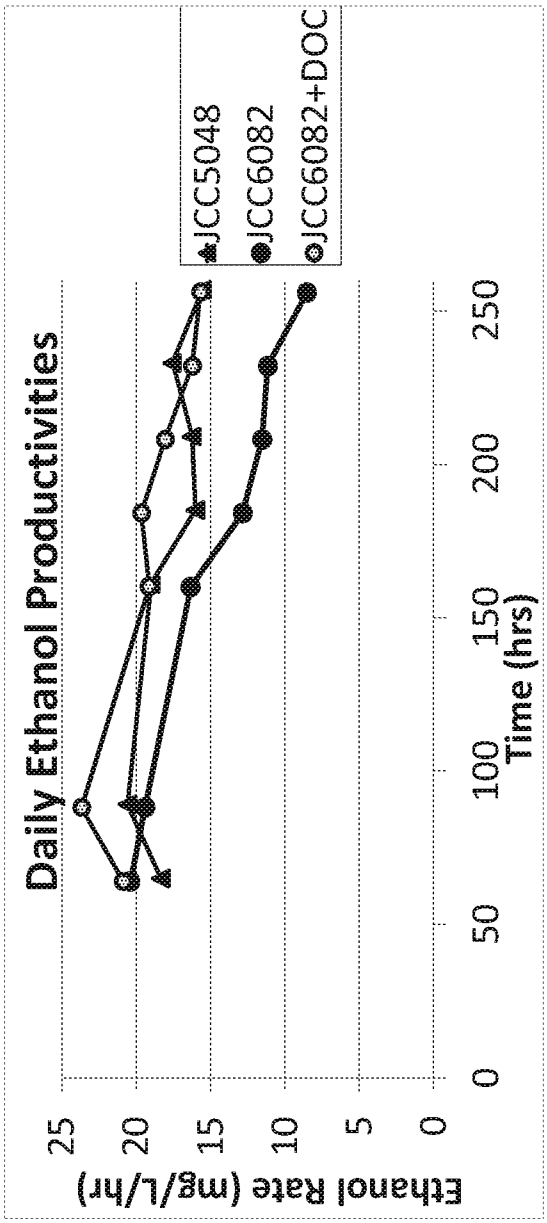
Daily Ethanol Productivities
JCC5048
JCC6082
JCC6082+DOC
Ethanol Rate (mg/L/hr)
25
20
15
10
5
0
0
50
100
150
200
250
Time (hrs)

Figure 22