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(54) Title: GENETICALLY ENGINEERED CYANOBACTERIA

(57) Abstract: The disclosed embodiments provide cyanobacteria spp. that have been genetically engineered to have increased production of carbon-based products of interest. These genetically engineered hosts efficiently convert carbon dioxide and light into carbon-based products of interest such as long chained hydrocarbons. Several constructs containing polynucleotides encoding enzymes active in the metabolic pathways of cyanobacteria are disclosed. In many instances, the cyanobacteria strains have been further genetically modified to optimize production of the carbon-based products of interest. The optimization includes both up- regulation and down-regulation of particular genes.

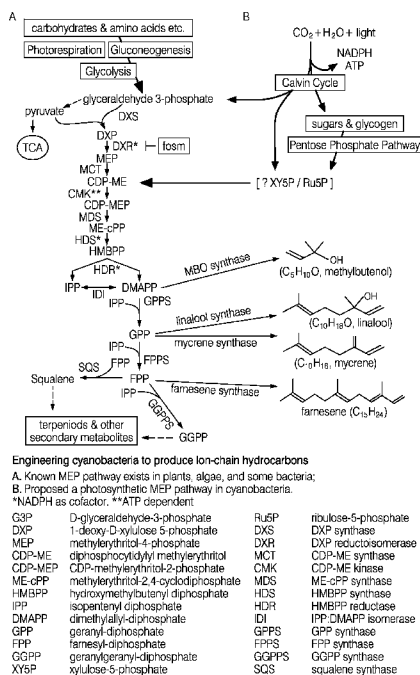


FIG. 4



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GENETICALLY ENGINEERED CYANOBACTERIA**TECHNICAL FIELD**

[0001] The present disclosure relates to compositions and methods for the production of carbon-based products of interest such as biofuels and high value chemicals by genetically engineered cyanobacteria hosts. The genetically engineered cyanobacteria hosts are optimized for use in production of carbon-based products of interest by strengthening endogenous metabolic pathways of cyanobacteria. In certain instances, competing metabolic pathways are down-regulated. Methods of making and using the genetically engineered cyanobacteria hosts are also described.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Patent Application Ser. No. 61/446,366, filed February 24, 2011, and U.S. Provisional Patent Application Ser. No. 61/522,685, filed August 11, 2011, the entire contents of each of which are incorporated herein by reference.

**STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT**

[0003] This invention was made with U.S. Government Support from the following agencies: USDA (Grant #SA1100114), NSF (Grant # CBET1133951), and NASA (Grant # NNX11AM03A). The U.S. Government has certain rights in this invention.

BACKGROUND

[0004] Many existing photoautotrophic organisms are poorly suited for industrial bioprocessing and have therefore not demonstrated commercial viability. Although aquatic photoautotrophs, such as cyanobacteria, may exhibit rapid growth rates and efficient

photosynthetic pathways, giving them tremendous potential for sustainable production of carbon-based products of interest from only CO₂, N₂, and sunlight, they have not yet been optimized for production. Such organisms typically require large amounts of water usage as well as time and energy to harvest biomass. Therefore, a need exists to modify existing photoautotroph hosts such that these drawbacks can be overcome.

SUMMARY

[0005] The present disclosure includes compositions and methods for the production of carbon based products of interest using genetically modified cyanobacteria such as *Anabaena* spp. In certain embodiments, the *Anabaena* spp. are *Anabaena* PCC7120, *Anabaena cylindrica* 29414, or *Anabaena variabilis* ATCC29413. In one aspect of the disclosure, an isolated *Anabaena* spp. is the ethanol producing *Anabaena* sp. PCC7120 (pZR672) strain deposited under ATCC accession number XXX or the linolool producing *Anabaena* sp. PCC7120 (pZR808) strain deposited under ATCC accession number XXX. Generally the *Anabaena* spp. is genetically engineered by expression of at least one recombinant polynucleotide expression construct comprising an enzyme capable of increasing production of a carbon based product of interest.

[0006] The carbon based product of interest may be ethanol or linolool. In many embodiments, the MEP pathway of the *Anabaena* spp. is up-regulated by modifying at least one gene responsible for control of the MEP pathway in the *Anabaena* spp. Photosynthesis of the *Anabaena* spp. may also be increased through genetic modification. For example, a polynucleotide expression construct comprising a nucleotide sequence encoding RuBisCo and/or RuBisCo activase is contemplated.

[0007] In certain embodiments, the *Anabaena* spp. is further genetically modified to produce enzymes capable of increasing specific production of ethanol or linolool. For example, in embodiments that specifically produce ethanol, the *Anabaena* spp. may be genetically engineered to produce decarboxylase (PDC) or alcohol dehydrogenase (ADH). In embodiments

specifically producing linolool, the *Anabaena* spp. may be genetically engineered to produce linolool synthase.

[0008] A disclosed method includes producing a genetically engineered *Anabaena* spp. capable of making a carbon based product of interest by introducing a recombinant enzyme into the *Anabaena* spp, wherein the recombinant enzyme can participate in the *Anabaena* spp's natural metabolic pathway, and modifying at least one competing metabolic pathway to increase production of the carbon based product of interest. In one disclosed aspect, the *Anabaena* spp. is the ethanol producing *Anabaena* sp. PCC7120 (pZR672) strain deposited under ATCC accession number XXX or the linolool producing *Anabaena* sp. PCC7120 (pZR808) strain deposited under ATCC accession number XXX. The natural metabolic pathway may be the MEP pathway or the photosynthetic pathway and the carbon based product of interest may be ethanol or linolool.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 demonstrates the presumptive cyanobacterial carbon metabolic pathways for production of biofuels and high value chemicals.

[0010] FIG. 2 demonstrates the modified cyanobacterial carbon metabolic pathway for production of ethanol.

[0011] FIG. 3 is ethanol productivity in genetically engineered *Anabaena* as measured by HPLC.

[0012] FIG. 4 shows (A)the known MEP pathway as it exists in plants, algae and some bacterial and (B)the proposed synthetic pathway in cyanobacteria.

[0013] FIG. 5 shows metabolic pathway for photosynthetic production of sucrose.

[0014] FIG. 6 shows (B) linalool production in genetically engineered *Anabaena* as measured by GC/MS and (C) native production of long chain alkanes/alkenes in wild-type *Anabaena* sp. PCC7120.

[0015] FIG. 7 shows mass spectra for linalool (C₁₀H₁₈O) standard.

[0016] FIG. 8 shows mass spectra for linalool produced by engineered *Anabaena*.

[0017] FIG. 9 shows hydrocarbons produced by *Anabaena cylindrica* 29414.

[0018] FIG. 10 shows engineering N₂-fixing cyanobacteria to produce urea using solar energy.

[0019] FIG. 11 demonstrates sucrose produced by *Anabaena* sp. PCC7120.

[0020] FIG. 12 illustrates a LinS gene integrated to *Anabaena* chromosome at loci A and B.

[0021] FIG. 13 shows a table of the MEP pathway genes in cyanobacteria.

DETAILED DESCRIPTION

[0022] For describing invention herein, the exemplary embodiments in detail, it is to be understood that the embodiments are not limited to particular compositions or methods, as the compositions and methods can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which an embodiment pertains. Many methods and compositions similar, modified, or equivalent to those described herein can be used in the practice of the current embodiments without undue experimentation.

[0023] As used in this specification and the appended claims, the singular forms “a,” “an” and “the” can include plural referents unless the content clearly indicates otherwise. Thus, for example, reference to “a cytokine” can include a combination of two or more cytokines. The term “or” is generally employed to include “and/or,” unless the content clearly dictates otherwise.

[0024] As used herein, “about,” “approximately,” “substantially,” and “significantly” will be understood by person of ordinary skill in the art and will vary in some extent depending on the context in which they are used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, “about” and “approximately” will mean plus or minus $\leq 10\%$ of particular term and “substantially” and “significantly” will mean plus or minus $> 10\%$ of the particular term.

[0025] The term "polynucleotide" 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 internucleoside 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. An "isolated" polynucleotide 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.

[0026] Polynucleotides 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, internucleotide 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. In certain embodiments, the polynucleotides are modified such that they contain preferential codon sequence for the host.

[0027] The term "percent sequence identity" or "identical" in the context of polynucleotide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The term "substantial homology" or "substantial similarity," when referring to a polynucleotide, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another polynucleotide (or its complementary strand), there is nucleotide sequence identity in at least about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity.

[0028] A heterologous sequence is a sequence that is in a different position or in a different amount than what is found in nature, 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).

[0029] A recombinant molecule is a molecule, 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 operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature. In many embodiments, the recombinant molecule is an enzyme. 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. A coding sequence is considered "recombinant" if it contains an insertion, deletion or a point mutation introduced artificially, e.g., by human intervention.

[0030] Molecules are "operably linked" if there is a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an

action on the other part (e.g., transcription of the sequence). Thus, a polynucleotide is "operably linked to a promoter" when there is a functional linkage between a polynucleotide expression control sequence (such as a promoter or other transcription regulation sequences) and a second polynucleotide sequence (e.g., a heterologous polynucleotide), where the expression control sequence directs transcription of the polynucleotide.

[0031] An "expression vector" or "construct" refers to a series of polynucleotide elements that are capable of transporting the polynucleotide elements into the host and permitting transcription of a gene in a host cell. Most embodiments require that the host have activity of the gene product as a consequence of being genetically engineered with an expression vector. For example, if the expression vector includes polynucleotide elements encoding a gene for an enzyme, the enzyme should have enzymatic activity after the host is genetically engineered. Typically, the expression vector includes a promoter and a heterologous polynucleotide sequence that is transcribed. Expression vectors or constructs may also include, e.g., transcription termination signals, polyadenylation signals, and enhancer elements. Constructs may also include polynucleotides that make them temperature sensitive, antibiotic resistant, or chemically inducible. Expression vectors can replicate autonomously, or they can replicate by being inserted into the genome of the host cell. In exemplary embodiment, the construct encoding the desired enzyme is present on 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.

[0032] The term "recombinant host cell" or "engineered host cell" (or simply "host cell" or "host") refers to a cell into which a recombinant polynucleotide has been introduced. Recombinant polynucleotides can be used to transform a variety of hosts to produce a carbon-based product of interest. The host must be "competent to express," such that it provides a sufficient cellular environment for expression of endogenous and/or exogenous polynucleotides. 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. Photoautotrophic organism hosts include organisms

such as eukaryotic plants and algae, as well as prokaryotic cyanobacteria, green-sulfur bacteria, green non-sulfur bacteria, purple sulfur bacteria, and purple non-sulfur bacteria.

[0033] In embodiments, the engineered cell of the invention is an algae and/or cyanobacterial organism selected from the group consisting of *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*, *Balbiana*, *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*, *Chlamydoublepharis*, *Chlamydocapsa*, *Chlamydomonas*, *Chlamydomonopsis*, *Chlamydomyxa*, *Chlamydonephris*, *Chlorangiella*, *Chlorangiopsis*, *Chlorella*, *Chlorobotrys*, *Chlorobraxis*, *Chlorochytrium*, *Chlorococcum*, *Chlorogloea*, *Chlorogloeopsis*, *Chlorogonium*, *Chlorolobion*, *Chloromonas*, *Chlorophysema*, *Chlorophyta*, *Chlorosaccus*, *Chlorosarcina*, *Choricystis*, *Chromophyton*, *Chromulina*, *Chroococcidiopsis*, *Chroococcus*, *Chroodactylon*, *Chroomonas*, *Chroothece*, *Chrysamoeba*, *Chrysapsis*, *Chrysidiastrium*, *Chrysocapsa*, *Chrysocapsella*, *Chrysochaete*, *Chrysochromulina*, *Chrysococcus*, *Chrysocrinus*, *Chrysolepidomonas*, *Chrysolykos*, *Chrysonebula*, *Chrysophyta*, *Chrysopyxis*, *Chrysosaccus*, *Chrysosphaerella*, *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, Gloecystis, Gloeodendron, Gloeomonas, Gloeoplax, Gloeotheca, Gloetila, 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, Microglana, 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, Pedastrum, 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, Polygonioclhoris, Polyepidomonas, Polyaenia, Polytoma, Polytomella, Porphyridium, Posteriochromonas, Prasinochloris, Prasinocladus, Prasinophyta, Prasiola, Prochlorophyta, 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, Styloxyis, Stylosphaeridium, Surirella, Sykidion, Symploca, Synechococcus, Synechocystis, Synedra, Synochromonas, Synura, Tabellaria, Tabularia, Teilingia, Temnogametum, Tetmemorus, Tetrachlorella, Tetracyclus, Tetradesmus, Tetraedriella, Tetraedron, Tetraselmis, Tetraspora, Tetrastrum, Thalassiosira, Thamniochaete, Thermosynechococcus, 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. In yet other related embodiments, the engineered cell provided by the invention is derived from a *Chloroflexus, Chloronema, Oscillochloris, Heliothrix, Herpetosiphon, Roseiflexus, and Thermomicrobium cell*; a green sulfur bacteria selected from: *Chlorobium, Clathrochloris, and Prosthecochloris*; a purple sulfur bacteria is selected from: *Allochromatium, Chromatium, Halochromatium, Isochromatium, Marichromatium, Rhodovulum, Thermochromatium, Thiocapsa, Thiorhodococcus, and Thiocystis*; a purple non-sulfur bacteria is selected from: *Phaeospirillum, Rhodobaca, Rhodobacter, Rhodomicrobium, Rhodopila, Rhodopseudomonas, Rhodothalassium, Rhodospirillum, Rhodovibrio, and Roseospira*; an aerobic chemolithotrophic bacteria selected from: nitrifying bacteria. *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., Thermoithrix sp.*; obligately chemolithotrophic hydrogen bacteria, *Hydrogenobacter sp.*, iron and manganese-oxidizing and/or depositing bacteria, *Siderococcus sp.*, and magnetotactic bacteria, *Aquaspirillum sp.*; an archaeobacteria selected from: methanogenic archaeobacteria, *Methanobacterium sp., Methanobrevibacter sp., Methanothermus sp., Methanococcus sp., Methanomicrobium sp., Methanospirillum sp., Methanogenium sp., Methanosarcina sp., Methanolobus sp., Methanotherix sp., Methanococcoides sp., Methanoplanus sp.*; extremely thermophilic sulfur-Metabolizers such as *Thermoproteus sp., Pyrodictium sp., Sulfolobus sp., Acidianus sp., Bacillus subtilis, Saccharomyces cerevisiae, Streptomyces sp., Ralstonia sp., Rhodococcus sp., Corynebacteria sp., Brevibacteria sp., Mycobacteria sp.*, and oleaginous yeast; and extremophile selected from *Pyrolobus fumarii; Synechococcus lividis, mesophiles, psychrophiles, Psychrobacter, insects,*

Deinococcus radiodurans, *piezophiles*, *barophiles*, hypergravity tolerant organisms, hypogravity tolerant organisms, vacuum tolerant organisms, tardigrades, insects, microbes seeds, dessicant tolerant anhydrobiotic organisms, xerophiles, *Artemia salina*, nematodes, microbes, fungi, lichens, salt tolerant organisms halophiles, halobacteriaceae, *Dunaliella salina*, pH tolerant organisms, alkaliphiles, *Natronobacterium*, *Bacillus firmus* OF4, *Spirulina* spp., *acidophiles*, *Cyanidium caldarium*, *Ferroplasma* sp., anaerobes, which cannot tolerate O₂, *Methanococcus jannaschii*, microaerophils, which tolerate some O₂, *Clostridium*, aerobes, which require O₂, gas tolerant organisms, which tolerate pure CO₂, *Cyanidium caldarium*, metal tolerant organisms, metalotolerants, *Ferroplasma acidarmanus* *Ralstonia* sp CH34.

[0034] In certain embodiments, the host is *Nostoc punctiforme* ATCC29133. In many embodiments, the host is an *Anabaena* spp of cyanobacterium. *Anabaena* provides several advantages above the cyanobacteria currently being genetically modified to produce carbon based products of interest. For example, *Anabaena* is capable of fixing its own N₂ for growth using heterocysts using only solar energy and water, allowing for less investment for growth. In one embodiment, the host is *Anabaena* PCC7120 (*Anabaena* 7120). In another embodiment, the host is *Anabaena cylindrica* 29414. In yet another embodiment, the host is *Anabaena variabilis* ATCC29413.

[0035] "Carbon-based products of interest" include alcohols such as ethanol, propanol, methylbutenol, linalool, geraniol, isopropanol, butanol, butanetriol, menthol, fatty alcohols, fatty acid esters, wax esters; hydrocarbons (alkanes/alkenes) such as propane, hexane, octane/octane, squalane, myrcene, decene, pinene, farnesene, limonene, 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, .epsilon.-caprolactone, isoprene, caprolactam, rubber; commodity chemicals such as lactate, Docosaheptaenoic acid (DHA), 3-hydroxypropionate, amino acids such as lysine, serine, aspartate, and aspartic acid, sorbitol, ascorbate, ascorbic acid, isopentenol, lanosterol, omega-3 DHA, itaconate, 1,3-butadiene, ethylene, propylene, succinate, citrate, citric acid, sucrose, glutamate, malate, 3-hydroxypropionic acid (HPA), lactic acid, THF, gamma butyrolactone, pyrrolidones, hydroxybutyrate, glutamic acid, levulinic acid, acrylic acid, malonic acid; specialty chemicals

including carotenoids such as lycopene, astaxanthin, β -carotene, and canthaxanthin, 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, i.e. any fuel with one or more hydrocarbons, one or more alcohols, one or more fatty esters or a mixture thereof that derives from a biological source industrial and specialty chemicals, as intermediates used to make additional products, such as nutritional supplements, nutraceuticals, polymers, paraffin replacements, personal care products and pharmaceuticals.

[0036] In various embodiments, polynucleotides encoding enzymes are introduced into the host cell such that expression of the enzyme by the host under certain conditions results in increased production of a carbon-based product of interest. In certain cases, introduction takes place through transformation of the host. “Increased production” or “up-regulation” of a carbon-based product of interest includes both augmentation of native production of the carbon-based product of interest as well as production of a carbon-based product of interest in an organism lacking native production. For example, in some instances production will be increased from a measureable initial value whereas in other instances the initial value is zero.

[0037] A recombinant expression construct for transformation of a host cell and subsequent integration of the gene(s) of interest is prepared by first isolating the constituent polynucleotide sequences. In some embodiments, the gene(s) of interest are homologously integrated into the host cell genome. In other embodiments, the genes are non-homologously integrated into the host cell genome. Generally, constructs containing polynucleotides are introduced into the host cell using a standard protocol, such as the one set out in Golden SS et al. (1987) “Genetic engineering of the Cyanobacteria chromosome” *Methods Enzymol* 153: 215-231 and in S.S. Golden and L.A. Sherman, *J. Bacteriol.* 158:36 (1984), incorporated herein by reference. The particular procedure used to introduce the genetic material into the host cell for expression is not particularly critical. Any of the well-known procedures for introducing heterologous polynucleotide sequences into host cells can be used. In certain embodiments, only a single copy of the heterologous polynucleotide is introduced. In other embodiments, more than

a single copy, such as two copies, three copies or more than three copies of the heterologous polynucleotide is introduced. As is understood by the skilled artisan, multiple copies of heterologous polynucleotides may be on a single construct or on more than one construct.

[0038] In exemplary embodiments, the disclosed polynucleotides are operably connected to a promoter in the construct. As is understood in the art, a promoter is segment of DNA which acts as a controlling element in the expression of that gene. In one embodiment, the promoter is a native *Anabaena* promoter. For example, the promoter may be an *Anabaena* Pnir promoter such as the one described in Desplancq, D2005, Combining inducible protein overexpression with NMR-grade triple isotope labeling in the cyanobacterium *Anabaena* sp. PCC 7120. *Biotechniques*. 39:405-11 (SEQ ID NO. 1) or one having sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 1. The promoter may also be an *Anabaena* psbA promoter (SEQ ID NO. 2), Prbc_L promoter (SEQ ID NO. 3) and/or *E. coli* P_{tac} promoter (SEQ ID NO. 4) (Elhai, J. 1993. Strong and regulated promoters in the cyanobacterium *Anabaena* PCC 7120. *FEMS Microbiol Lett.* 114(2):179-84) or one having sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 2, SEQ ID NO. 3, or SEQ ID NO. 4. In some embodiments, the promoter is a combined dual promoter, i.e. a promoter containing more than one of the above.

[0039] In some embodiments, the gene of interest is transiently introduced into the host cell through use of a plasmid or shuttle vector. In other embodiments, the gene of interest is permanently introduced into the chromosome of the host cell. Chromosomal integration techniques are known to the skilled artisan and have been described in Zhou and Wolk, 2002, Identification of an Akinete Marker Gene in *Anabaena variabilis*, *J. Bacteriol.*, 184(9):2529-2532. Briefly, the gene of interest is fused to a promoter and then subcloned into an integration vector. This construct is introduced into the host cell for double homologous recombination at specific loci on the host cell chromosome. In many embodiments, homologous recombination takes place at two loci of the host cell chromosome. The recombinant cells can be selected by monitoring loss of a conditional lethal gene, such as *sacB*. Further diagnostic verification by the

polymerase chain reaction can be performed. In many embodiments, the gene of interest will be inserted into the chromosome at the site of a gene that is desired to be deleted or inactivated.

[0040] After the host is genetically modified, the host is generally incubated under conditions suitable for production of the carbon-based product of interest. Culture conditions for various hosts are well documented in the literature. Typically, when the host is *Anabaena*, the host cell will be grown in a photoautotrophic liquid culture in BG-11 media, with an 1L/min air sparge rate and a pH set point of 7.5, controlled via sparging with CO₂, and the temperature maintained at 30°C.

[0041] In many embodiments, strain engineering techniques such as directed evolution and acclimation will be used to improve the performance of various host cells. Strain engineering is known in the art (Hughes, S.R., K.M. Bischoff, W.R. Gibbons, S.S. Bang, R. Pinkelman, P.J. Slininger, N. Qureshi, S. Liu, B.C. Saha, J.S. Jackson, M.C. Cotta, J.O. Rich, and J. Javers. 2011. Random UV-C Mutagenesis of *Scheffersomyces* (formerly *Pichia*) *stipitis* NRRL Y-7124 to Improve Anaerobic Growth on Lignocellulosic Sugars. *J. Ind. Microbiol. Biotechnol.* DOI 10.1007/x 10295-011-1012-x; Bock, S.A., Fox, S.L. and Gibbons. W.R. 1997. Development of a low cost, industrially suitable medium for production of acetic acid from glucose by *Clostridium thermoaceticum*. *Biotechnol. Applied Bioch.* 25:117-125; Gibbons, W.R., N. Pulscher, and E. Ringquist. 1992. Sodium meta bisulfite and pH tolerance of *Pleurotus sajor caju* under submerged cultivation. *Appl. Biochem. Biotechnol.* 37:177-189.

[0042] As host cells generally possess complex regulatory systems for traits such as product tolerance, productivity, and yield, directed evolution and screening is often used to create global genome-wide alterations needed to develop strains with desired industrial characteristics. Certain embodiments will use directed evolution under elevated linalool concentrations, as well as fluctuating temperature, pH, and CO₂/O₂ levels to generate stable, heritable genetic improvements in product tolerance, productivity, yield, and robustness to process conditions.

[0043] A. Ethanol

[0044] In one embodiment, the host cell is genetically engineered to increase production of ethanol through transformation with an expression vector containing polynucleotides encoding ethanol producing enzymes. As used herein, an ethanol producing enzyme is an enzyme active in the end production of ethanol from a precursor molecule in a metabolic pathway. The polynucleotide encodes pyruvate decarboxylase (SEQ ID NO. 5) and/or alcohol dehydrogenase (SEQ ID NO. 6) in exemplary embodiments. Embodiments also include enzymes having sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 5 and SEQ ID NO. 6. The host is genetically engineered with polynucleotides encoding one or both enzymes. In many embodiments, host cells are engineered to express both enzymes. Known sources of polynucleotides encoding pyruvate decarboxylase and alcohol dehydrogenase exist. For example, the nucleic acid encoding the enzymes may be from organisms such as *Zymomonas mobilis*, *Zymobacter paimae*, or *Saccharomyces cerevisiae* (Ingram LO, Conway T, Clark DP, Sewell GW, Preston JF. 1987. Genetic engineering of ethanol production in *Escherichia coli*. Appl Environ Microbiol. 53(10):2420-5). Any pyruvate decarboxylase (pdc) gene capable of expression in the host may be used in with the disclosed embodiments. In some embodiments, the pdc gene is the *Zymomonas mobilis* pdc gene. In these embodiments, the pdc gene is often obtained from the *Zymomonas mobilis* plasmid pLOI295. In other embodiments, the pdc gene is from *Zymobacter paimae*. The NCBI accession number for the complete pdc protein sequence from *Zymobacter paimae* is AF474145. Similarly, any alcohol dehydrogenase (adh) gene capable expression in the host may be used with the disclosed embodiments. In some embodiments, the adh gene is the *Zymomonas mobilis* adhII gene. In these embodiments, the adh gene is often obtained from the *Zymomonas mobilis* plasmid pLOI295.

[0045] Polynucleotides encoding genes such as *omrA*, *lmrA*, and *lmrCD*, which increase the ability of the host to handle commercially relevant amounts of ethanol, may be included on the same or a different vector as the polynucleotides encoding the pdc and adh genes. Bourdineaud JP, Nehmé B, Tesse S, Lonvaud-Funel A. 2004. A bacterial gene homologous to ABC transporters protect *Oenococcus oeni* from ethanol and other stress factors in wine. *Int. J. Food Microbiol.* 92(1):1-14. For example, in some embodiments, the expression vector comprising the pdc/adh genes further comprises the *omrA* gene. In other embodiments, the

expression vector comprising the *pdc/adh* genes further comprises the *lmrA* gene. In yet other embodiments, the expression vector comprising the *pdc/adh* genes further comprises the *lmrCD* gene. And in still further embodiments, the expression vector comprising the *pdc/adh* genes further comprises polynucleotides encoding the *omrA*, *lmrA*, and *lmrCD* genes.

[0046] In host cells producing increased ethanol, the synthesis of pyruvate is additionally up-regulated in certain embodiments. In these embodiments, phosphoglycerate mutase, enolase, and/or pyruvate kinase, are over-expressed. A construct containing genes of one or more of the above enzymes is designed using genes from *Z. mobilis* and *S. cerevisiae*. The construct is then used to genetically engineer a host.

[0047] Ethanol producing *Anabaena* sp. PCC7120 (pZR672) strain was deposited at the American Type Culture Collection on February 24, 2012, and given accession number XXXX.

[0048] B. Sucrose

[0049] In yet another embodiment, the host cell is engineered to increase the production and excretion of sucrose through transformation with an expression vector containing polynucleotides encoding sucrose producing enzymes. As used herein, a sucrose producing enzyme is an enzyme active in the end production of sucrose from a precursor molecule in a photosynthetic pathway. In these embodiments, a polynucleotide encoding sucrose-phosphate synthase (SPS) and/or sucrose-phosphate phosphatase (SPP) is introduced into the host cell and expressed such that the host cell increases its production of sucrose. Known sources of SPS and SPP exist and any SPS or SPP gene capable of expression may be used with the disclosed embodiments. For example, polynucleotide encoding SPS and SPP may be from organisms such as sugar beet and sugar cane such as those in SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9. In other embodiments, the polynucleotides have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 7, SEQ ID NO. 8, and SEQ ID NO. 9. In an alternative embodiment, the polynucleotide encoding SPS and is from cyanobacteria such as *Synchocystis*, *Anabaena*, or the like. Polynucleotides of SPS from cyanobacteria are shown in SEQ ID NO. 10 and SEQ ID NO. 11. In certain embodiments, SPS

polynucleotides have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO.10 and SEQ ID NO. 11.

[0050] In exemplary embodiments, the expression vector encoding SPS and/or SPP includes a promoter. For example, in some embodiments, the expression vector includes an *Anabaena* PpsbA promoter. In this embodiment the expression vector may be shuttle vector pRL489, such as the one described in Elhai J 1993 Strong and regulated promoters in the cyanobacterium *Anabaena* PCC7120. *FEMS Microbiol. Lett.* 114(2): 179-84.

[0051] In many embodiments where sucrose production has been increased, intracellular sucrose concentrations are reduced by over-expression of sucrose exporter genes. A sucrose exporter gene is a gene encoding a polypeptide involved in the transport of sucrose out of the cell. An example sucrose exporter gene includes the sucrose exporter gene from maize, i.e. ZmSUT1 (Slewinski *et al.*, 2009. Sucrose transporter1 functions in phloem loading in maize leaves. *J. Exp. Bot.* 60 (3):881-892). A sucrose exporter gene is demonstrated by SEQ ID NO. 12. In some embodiments, the sucrose exporter genes have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 12. The host in certain embodiments is genetically engineered with a sucrose exporter gene which is on the same construct as SPS and/or SPP. In other embodiments, the sucrose exporter genes may be from sugarcane and cloned into a separate expression vector or integrated into the chromosome of the host cells. Reinders A, Sivitz AB, Hsi A, Grof CP, Perroux JM, Ward JM. 2006. Sugarcane ShSUT1: analysis of sucrose transport activity and inhibition by sucralose. *Plant Cell Environ.* 29(10):1871-80 demonstrates the sucrose exporter gene of SEQ ID NO. 13. In exemplary embodiments, the sucrose exporter genes have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 13

[0052] C. Urea

[0053] Additionally, other urea cycle pathway intermediates are up-regulated and non-urea producing metabolic pathways are down-regulated or blocked in exemplary embodiments. For example, in one embodiment the urea cycle genes, i.e. CPS-1, OTC, ASS, and AS, are up-

regulated. Polynucleotides encoding the genes are operably connected to an *Anabaena* PglA promoter and the host cell is genetically engineered with the construct.

[0054] D. Long Chain Alkanes

[0055] In still another embodiment, host cells are engineered to increase production of long chain hydrocarbons such as alkanes/alkenes, i.e. C8-C18. In many embodiments with increased production of long chain hydrocarbons, secretion of the long chain hydrocarbons is also increased. *Anabaena* is innately capable of producing and secreting long-chain alkanes/alkenes. Long chain alkanes/alkenes can be produced in *Anabaena* from both the fatty acid pathway and the MEP pathway. In the fatty acid pathway, acyl-ACP reductase (AR) combined with aldehyde decarbonylase (AD) convert fatty acid to alkanes/alkenes Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. 2010. Microbial biosynthesis of alkanes. *Science*. 329(5991):559-62. In embodiments where host cells are engineered to increase production of long chain alkanes, the host cell is genetically engineered with a polynucleotide encoding AR and/or AD. Known sources of AR and AD exist in many cyanobacteria and any AR and AD gene capable of expression may be used with the disclosed embodiments. In many embodiments, the AR and/or AD genes are native *Anabaena* genes, i.e. native AR and/or AD are over-expressed. For example, in one embodiment the AR/AD genes will be from *Anabaena cylindrica* 29414 such as those demonstrated by SEQ ID NO. 14 and SEQ ID NO. 15. In other embodiments, the AR and AD genes have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 14 and SEQ ID NO. 15.

[0056] E. Long-chain hydrocarbons from Isoprenoid Biosynthesis Pathway

[0057] In still another embodiment, the host cell is engineered to increase the production of carbon-based products of interest from the native isoprenoid biosynthesis pathway, i.e. the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. In many embodiments, excretion of the carbon-based products of interest is also increased. DMAPP and IPP, the early precursors for many carbon-based products of interest are made through MEP pathway in *Anabaena*. In heterotrophic organisms, DMAPP and IPP are made from precursors mainly derived from

glucose through gluconeogenesis. However, as demonstrated in FIG. 4 photosynthetic organisms produce DMAPP and IPP from precursors directly synthesized from CO₂ via the Calvin cycle and perhaps also from photorespiration. Cyanobacteria, in addition to initiating the MEP pathway via glyceraldehyde-3-phosphate (G3P) and pyruvate, can use phosphorylated sugars directly from the Calvin cycle as precursors for entering into the MEP pathway. Due to their higher photosynthetic efficiency and greater innate MEP pathway flux for making DMAPP and IPP precursors, cyanobacteria, such as *Anabaena* are especially suited for engineering production of excreted carbon-based products of interest. Therefore, genetically engineering photosynthetic organisms such as *Anabaena* to produce MEP pathway carbon-based products of interest has greater advantages than genetically engineering heterotrophic organisms.

[0058] In some embodiments, components of the MEP pathway are up-regulated to manipulate the DMAPP and IPP pool so as to maximize production of carbon-based products of interest. This up-regulation is achieved through transformation of the host by an expression vector with polynucleotides containing one or more of the eight genes of the MEP pathway. FIG. 4 and FIG. 13 show the individual components of the MEP pathway. The genes responsible for the MEP pathway include *dxs*, *dxr*, *mct*, *cmk*, *mds*, *hds*, *hdr*, and *idi*. In many cases, the MEP pathway polynucleotide expression may be constructed as a synthetic operon. This operon is fused to an *Anabaena* *psbA* promoter in pZR807 (a pNIR derivative shuttle vector) in many embodiments. In certain embodiments, the *dxr*, *hds*, and *hdr* are from *Synechocystis* sp. PCC6803. In *Synechocystis*, the corresponding genes are *sll0019*, *slr2136*, and *slr0348* respectively. In another embodiment, DXS will be overexpressed. Kuzuyama T, Takagi M, Takahashi S, Seto H. 2000. Cloning and characterization of 1-deoxy-D-xylulose 5-phosphate synthase from *Streptomyces* sp strain CL190, which uses both the mevalonate and nonmevalonate pathways for isopentenyl diphosphate biosynthesis. *J. Bacteriol.* 182(4):891-7, Cordoba E, Salmi M, León P. 2009. Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. *J Exp Bot.* 60(10):2933-43, Alper H, Fischer C, Nevoigt E, Stephanopoulos G. 2005. Tuning genetic control through promoter engineering. *Proc. Natl. Acad. Sci. U S A.* 102:12678-83, Alper H, Stephanopoulos G. 2008. Uncovering the gene knockout landscape for improved lycopene production in *E. coli*. *Appl. Microbiol. Biotechnol.* 78:801-10. In this embodiment, to overexpress DXS, the DXS gene (*alr0599*) from *Anabaena* will be PCR

amplified with primers containing restriction sites and a ribosome binding site. The resulting PCR product will be fused to a nitrate-inducible promoter P_{nir} and cloned into pZR807, a shuttle plasmid that can replicate both in *E. coli* and *Anabaena*. This construction will be introduced into *Anabaena* for overexpression of DXS.

[0059] The genes of the MEP pathway are generally placed into the operon in the pathway order, although this is not required. The genes may be flanked with restriction nuclease sites non-native to the applicable genes to make insertion and deletion of specific genes more convenient. When the restriction sites are intended to allow removal of a portion of the operon and replacement with another sequence, different restriction enzyme sites are used on each side of the portion of the operon. When the restriction sites are intended to allow removal of a portion of the operon and not be replaced, the same restriction nuclease site exists on both sides. In most embodiments, restriction nuclease sites are engineered to produce sticky-ends. Polynucleotide sequences for individual genes have engineered ribosome binding sites in many embodiments. In some instances, the genes additionally include spacer sequences for enhancing translation of target genes.

[0060] a. Linalool (C₁₀H₁₈O)

[0061] Linalool (C₁₀H₁₈O) is a carbon-based product of interest produced from the MEP pathway where the universal isoprenoid intermediate geranyl diphosphate (GPP) is converted to linalool by linalool synthase (LinS) (see FIG. 4). In these embodiments, host is genetically engineered with a polynucleotide encoding LinS such that the host cell has up-regulated production of linalool. Known sources of LinS genes exist and any LinS gene capable of being expressed may be used with the disclosed embodiments. For example, polynucleotide encoding LinS may be from a Norway Spruce. In many embodiments, the polynucleotide encoding LinS is not native to *Anabaena*. LinS genes such as CbLinS, McLinS, and LaLinS are well studied and contemplated for use in the disclosed embodiments.

Table 1. Genes required for linalool production in engineering cyanobacteria					
	Gene name	Accession No.	Km (μM)	Organism	References
linalool synthase	<i>LaLINS</i>	DQ263741	47.4	<i>Lavandula angustifolia</i>	Landmann et al., 2007
	<i>Mc Lis</i>	AY083653	25	<i>Mentha citrata</i>	Crowell et al., 2002
	<i>CbLis</i>	U58314	0.9	<i>Clarkia breweri</i>	Pichersky et al., 1995 Dudareva et al., 1996

[0062] In exemplary embodiments, the expression vector encoding LinS includes a promoter. For example, in some embodiments, the expression vector includes an *Anabaena* Pnir promoter. In this embodiment the expression vector may be a shuttle vector pZR807.

[0063] In many embodiments, a host cell is genetically engineered with both polynucleotide encoding genes of the MEP pathway as well as LinS. This transformation may include a single expression vector or multiple expression vectors. In other embodiments, a LinS gene is fused to a promoter and then subcloned into an integration vector and this resulting construction pLinS is then introduced into the host cell for double homologous recombination. The double recombinants are then selected by loss of a conditional lethal gene such as *sacB*.

[0064] Linalool producing *Anabaena* sp. PCC7120 (pZR808) strain was deposited at the American Type Culture Collection on February 24, 2012, and given accession number XXXX.

[0065] b. Methylbutenol ($C_5H_{10}O$)

[0066] Another carbon-based product of interest produced by an intermediate product from the MEP pathway, i.e. DMAPP, is methylbutenol (MBO). Methylbutenol is produced in the MEP pathway when DMAPP is converted to methylbutenol by methylbutenol synthase (MboS). In these embodiments, host cell is genetically engineered with a polynucleotide encoding MboS such that the host cell has up-regulated production of methylbutenol. Known sources of MboS exist and any MboS gene capable of being expressed may be used with the disclosed embodiments. In certain embodiments, the polynucleotide encoding MboS is from *Pinus sabiniana* and listed as below. Gray DW, Breneman SR, Topper LA, Sharkey TD. 2011, Biochemical characterization and homology modeling of methylbutenol synthase and implications for understanding hemiterpene synthase evolution in plants. J Biol Chem. 286(23):20582-90. SEQ ID NO. 16. In other embodiments, MboS have sequence identity of about 76%, 80%, 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% to SEQ ID NO. 16.

[0067] In many embodiments, a host cell is genetically engineered with both polynucleotide encoding genes of the MEP pathway as well as MboS. This transformation may include a single expression vector or multiple expression vectors.

[0068] c. Myrcene (C₁₀H₁₆)

[0069] Yet another carbon-based product of interest produced from an intermediate of the MEP pathway is myrcene. Myrcene is produced in the MEP pathway where the universal isoprenoid intermediate geranyl diphosphate (GPP) is converted to myrcene by myrcene synthase (MyrS) Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Fäldt J, Miller B, Bohlmann J. 2003. (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell*. 15(5):1227-41. Martin DM, Fäldt J, Bohlmann J. 2004. Functional characterization of nine Norway Spruce TPS genes and evolution of gymnosperm terpene synthases of the TPS-d subfamily. *Plant Physiol*. 135(4):1908-27. Lijima Y, Davidovich-Rikanati R, Fridman E, Gang DR, Bar E, Lewinsohn E, Pichersky E. 2004. The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant Physiol*. 136(3):3724-36. No MyrS gene is founded in cyanobacterial genomes. In these embodiments, host is genetically engineered with a polynucleotide encoding MyrS such that the host cell has increased production of myrcene. Known sources of MyrS exist and any MyrS gene capable of being expressed may be used with the disclosed embodiments. In many embodiments, the polynucleotides encoding MyrS may be chosen from the organisms listed in the following table:

Table 2. Myrcene synthase gene required for engineering cyanobacteria to produce myrcene

	Gene Name	Accession No.	Organism
Myrcene synthase (MyrS)	<i>Ag.2.</i>	U87908	<i>Abies grandis</i>
	<i>Amale20</i>	AA041726	<i>Antirrhinum majus</i>
	<i>PaTPs-Myr</i>	AY473626	<i>Norway Spruce</i>

	<i>MyS</i>	AAV63791	<i>Ocimum basilicum</i>
	<i>Ama0c15</i>	AY195608	<i>Snapdragon</i>

[0070] In many embodiments, a host cell is genetically engineered with both polynucleotide encoding genes of the MEP pathway as well as MyrS. This transformation may include a single expression vector or multiple expression vectors.

[0071] d. Farnesene (C₁₅H₂₄)

[0072] And still another carbon based product of interest produced by MEP pathway is farnesene. Farnesene is produced in the MEP pathway by conversion of geranyl-diphosphate (GPP) to farnesyl-diphosphate (FPP) by FPP synthase (FPPS). Subsequently, FPP is converted to farnesene by farnesene synthase (FarS) Maruyama T, Ito M, Honda G. 2001. Molecular cloning, functional expression and characterization of (E)-beta farnesene synthase from *Citrus junos*. *Biol. Pharm. Bull.* 24:1171-5 and Picaud S, Brodelius M, Brodelius PE. 2005. Expression, purification and characterization of recombinant (E)-beta-farnesene synthase from *Artemisia annua*. *Phytochemistry*. 66(9):961-7. In *Anabaena*, only a putative FPPS gene exists and no FarS gene is found. In these embodiments, host cell is genetically engineered with a polynucleotide encoding FPPS and FarS such that the host cell has increased production of farnesene. Known sources of FPPS and FarS exist and any FPPS or FarS gene capable of being expressed may be used with the disclosed embodiments. In many embodiments, the polynucleotides encoding FPPS and FarS are chosen from the organisms listed in the following table:

Table 3. Genes required for engineering cyanobacteria to produce farnesene

	Gene Name	Accession No.	Organism
Farnesyl diphosphate synthase (FPPS)	<i>FDSI</i>	AY308477	<i>Artemisia tridentate</i>
	<i>TbFPPS</i>	AY158342	<i>Trypanosoma brucei</i>
	<i>FPS2</i>	NP_974565	<i>Arabidopsis thaliana</i>
	<i>ispA</i>	NP-414955	<i>E. coli K-12</i>

	<i>pFPS2</i>	U20771	<i>Lupinus albus</i>
Farnesene synthase (FarS)	<i>AFS1</i>	AY182241	<i>Malus domestica</i>
	<i>CJFS</i>	AF374462	<i>Citrus junos</i>
	<i>CmTpsDul</i>	EU158099	<i>Cucumis melo L.</i>
	<i>FS</i>	AY835398	<i>Artemisis annua</i>
	<i>PmeTPS4</i>	AY906867	<i>Pseudotsuga menziesii</i>

[0073] In certain embodiments, the FPPS and FarS will be from the same organism. In other embodiments, the constructs will include FPPS and FarS from different organisms. In many embodiments, a host cell is genetically engineered with both polynucleotide encoding genes of the MEP pathway as well as FPPS and FarS. This transformation may include a single expression vector or multiple expression vectors.

[0074] In most embodiments, production of carbon-based products of interest is further optimized. For example, photosynthesis is optimized and/or competing metabolic pathways are blocked or inactivated. Photosynthetic rates can be increased by the over-expression of RuBisCo and RuBisCo activase. Hudson GS, Evans JR, von Caemmerer S, Arvidsson YB, Andrews TJ. 1992. Reduction of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Content by Antisense RNA Reduces Photosynthesis in Transgenic Tobacco Plants. *Plant Physiol.* 98, 294-302 and Peterhansel C, Niessen M, Kebeish RM. 2008. Metabolic engineering towards the enhancement of photosynthesis. *Photochem. Photobiol.* 84:1317-23. In embodiments where host cells producing the carbon-based products of interest using CO₂ and H₂O as the starting material, the hosts are often additionally genetically engineered with polynucleotides encoding RuBisCo and RuBisCo activase.

[0075] When carbon-based products of interest are produced from the MEP pathway, glycogen synthesis, which competes with the MEP metabolic pathway in the host is down-regulated or blocked in many embodiments. Glycogen synthesis is down-regulated or blocked by the down-regulation or block of ADP-glucose pyrophosphorylase (ADP-GPPase) activity.

Pyruvate dehydrogenase (PDH) is additionally or alternatively blocked in these embodiments. GPP flux may be optimized by downregulating farnesyl-disphosphate synthase (FPPS). Additionally, in certain embodiments genes for the tolerance of a host cell to economically relevant concentrations of the carbon based product of interest are included. In embodiments where competing carbon pathways are blocked or partially inactivated, this may be done using any method known in the art. For example, enzymes in competing pathways can be knocked out or have their activity blocked or reduced. In certain embodiments, unmarked gene deletion created by double-crossover to delete target genes is used to delete *Anabaena* genes.

EXAMPLES

[0076] The invention may be further clarified by reference to the following Examples, which serve to exemplify some of the embodiments and not to limit the invention in any way. The experiments were performed using the methodology described below.

[0077] EXAMPLE 1.

[0078] Conjugation

[0079] Briefly, host cells are harvested by centrifugation and re-suspended in medium at a concentration of about $2-5 \times 10^8$ cells per ml. To one ml of this cell solution is added the appropriate construct to a final concentration of 2 $\mu\text{g/ml}$. Host cells are incubated in the dark for 8 hours followed by a 16 h light incubation prior to plating on media plates containing antibiotic. Plates are incubated under standard growth conditions (30°C light intensity of 100 $\mu\text{mol photons m}^{-2} \text{ S}^{-1}$). Antibiotic resistant colonies are chosen and the genetically modified host cells are grown, bubbling with air at 30°C and a light intensity of 100 $\mu\text{mol photons m}^{-2} \text{ S}^{-1}$ in liquid medium containing an appropriate antibiotic

[0080] EXAMPLE 2.

[0081] Culture Growth

[0082] Transgenic cyanobacter cultures will be grown in liquid BG-11 medium in a lighted shaker (Innova 44R, New Brunswick Scientific) at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. One week-old cultures will be used to re-inoculate 500 ml Erlenmeyer flasks containing 100 ml liquid BG11, which will then be incubated at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with a 24h lighting set. Heterotrophic cultures will be supplemented with 100 g L⁻¹ glucose. Samples will be collected at regular intervals and analyzed for product production, as well as chlorophyll content. Chlorophyll will be measured with a spectrophotometer.

[0083] EXAMPLE 3.

[0084] Ethanol Production

[0085] Both *pdc_{zm}* and *adhB_{zm}* coding regions, with an engineered optimized SD sequence (ribosome binding site) immediately upstream of their initiation codons were PCR amplified from pLOI295, which contains both *pdc_{zm}* and *adhB_{zm}* in an artificial operon. See Ingram LO *et al.* 1987 Genetic Engineering of Ethanol Production in *Escherichia coli*. *Appl. Environ. Microbiol.* 53(10):2420-5. The DNA fragment was fused to *Anabaena* nitrate inducible promoter (*nir*) in shuttle vector. See Desplancq, D. *et al.* 2005 Combining inducible protein overexpression with NMR-grade triple isotope labeling in the cyanobacterium *Anabaena* sp. PCC 7120. *Biotechniques.* 39:405-11 and Frias *et al.* 2000. Activation of the *Anabaena nir* operon promoter requires both NtcA (CAP family) and NtcB (LysR family) transcription factors. *Mol Microbiol.* 38:613-25. This construct, named pZR672, was introduced into *Anabaena* by conjugation. See Zhou, R. and Wolk, C.P. 2002. Identification of an akinete marker gene in *Anabaena variabilis*. *J Bacteriol.* 184:2529-32; Wolk, C.P. *et al.* 1984 Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria, *Proc Natl Acad Sci U S A.* 81:1561-5; and Zhou, R. and Wolk, C.P. 2003. A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J Biol Chem.* 278:19939-46. Genetically engineered hosts were selected in a nitrate-minus (AA/8 medium) Kan plate. Tests of ethanol production were done using well established protocols. Current ethanol productivity, as shown in FIG. 3 is about 13.8 mg/liter/h/1.0A₇₀₀.

[0086] EXAMPLE 4.

[0087] Sucrose Production

[0088] Both *sps* and *spp* coding regions, with an engineered optimized SD sequence (ribosome binding site) immediately upstream of their initiation codons will be PCR amplified from sugarcane/sugar beet cDNA. The DNA fragment will be fused to *Anabaena* nitrate inducible promoter (*nir*) in shuttle vector pNIR. This construct will be introduced into *Anabaena* by conjugation. See Wolk, C.P. et al. 1984 Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria, *Proc Natl Acad Sci U S A*. 81:1561-5. Genetically transformed *Anabaena* will be selected in a nitrate-containing (AA/8 N medium) Km plate. Antibiotic resistant colonies will be chosen and the genetically modified host cells will be grown, bubbling with air at 30 °C and a light intensity of 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in liquid medium containing appropriate antibiotic. HPLC tests of sucrose production by *Anabaena* sp. PCC7120 are demonstrated in FIG. 11.

[0089] Sucrose degradation will be reduced by blocking invertases and sucrose synthases (SuS) (see FIG. 5). Two genes, *alr0819* and *alr1521*, coding for *Anabaena* invertases and two genes, *all4985* and *all1059*, coding for sucrose synthases will be inactivated in a double crossover approach, such as the one demonstrated in Zhou, R., Wolk, C.P. 2003. A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J. Biol. Chem.* 278:19939-46. Phosphofructokinase (PFK) will also be down-regulated in certain embodiments. The genes coding for *Anabaena* PFK, *all7335* and *alr1919*, will be down-regulated or knocked out using a double crossover approach or through expression of the antisense gene. In one embodiment, one PFK gene will be knocked out, while the other will be down-regulated. In another embodiment, both PFK genes will be down-regulated.

[0090] EXAMPLE 5.

[0091] Urea Production.

[0092] a. Create a novel strain with more closely spaced heterocysts. It is known that overexpression of patA gene in *Anabaena* or inactivation of patN gene in *Nostoc punctiforme* led to more closely spaced single heterocysts, with an average vegetative cell interval of 3.2 cells (Meeks, J. C., E. L. Campbell, M. L. Summers, and F. C. Wong. 2002. Cellular Differentiation in the cyanobacterium *Nostoc punctiforme*. *Arch. Microbiol.* 178: 395 – 403; Liang J, Scappino L, Haselkorn R. 1992. The patA gene product, which contains a region similar to CheY of *Escherichia coli*, controls heterocyst pattern formation in the cyanobacterium *Anabaena* 7120. *Proc. Natl. Acad. Sci. U S A.* 89(12):5655-9)). A novel *Anabaena* will be created by combining over-expression of patA and inactivation of patN in *Anabaena*. This patA+patN- strain will serve as a model strain for further genetic modification to produce urea.

[0093] b. Manipulate nitrogen flux in patA+patN- strain. *Anabaena* will be engineered to convert surplus ammonia to urea. All 5 human homologous genes required for urea cycle are found in the *Anabaena* genome, as well as genes coding for urea transporters. The urea cycle's final reaction is arginase-catalyzed hydrolysis of arginine to yield urea and regenerate ornithine (FIG. 10). Initially an authentic arginase LeARG1 from tomato will be overexpressed in patA⁺patN⁻ strain and inactivate its urease Alr3666. Chen H, McCaig BC, Melotto M, He SY, Howe GA. 2004, Regulation of plant arginase by wounding, jasmonate, and the phytotoxin coronatine. *J. Biol. Chem.* 279(44):45998-6007. To overexpress these genes in *Anabaena*, the *Anabaena* PglA, a constitutively strong promoter that functions in both vegetative cells and heterocysts, will be fused to urea cycle genes and followed by over-expression of them in the patA⁺patN⁻ urease⁻LeARG⁺ strain.

[0094] c. Shut down the cyanophycin synthesis in patA⁺patN⁻urease⁻ LeARG⁺ strain. Cyanophycin synthesis will be blocked and fixed nitrogen will be redirected to excreted urea. A single gene, all3879, encoding cyanophycin synthetase will be knocked out by a double crossover approach (Zhou R, Wolk CP. 2003. A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J. Biol. Chem.* 278:19939-46).

[0095] The disclosed genetically engineered urea-producing *Anabaena* strains will be grown in a liquid N₂-medium (Bg11₀ medium which contains no combined nitrogen) in a lighted shaker (Innova 44R, New Brunswick Scientific) at 30°C and 150 μmol photons m⁻² s⁻¹. One

week-old cultures will be used to re-inoculate 4-liter Erlenmeyer flasks containing 1000 ml liquid BG11₀, which will then be incubated at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with a 24h lighting set. Samples will be collected at regular intervals (24h) and analyzed for urea production. Urea excreted in the culture fluid will be measured by HPLC. Results will be used to guide further genetic manipulations.

[0096] EXAMPLE 6.

[0097] Long Chain hydrocarbon Production and Isoprenoid Biosynthetic Pathway Product Production.

[0098] a. Linalool production

[0099] To engineer *Anabaena* to produce linalool, CbLinS, McLinS, and LaLinS (see Table 1) will be transferred into *Anabaena*. The coding region of the three genes, with N-terminal plastid targeted sequence deletion, was cloned immediately downstream of the engineered translation initiation sequence (Shine-Dalarno sequence) under a dual promoter (*Pnir/PsbA*) in shuttle vector pZR807, a pNIR derived plasmid that replicates in *Anabaena*. Each construct will be introduced into *Anabaena* by conjugation.

[00100] Transgenic *Anabaena* cultures will be grown in liquid BG-11 medium in a lighted shaker (Innova 44R, New Brunswick Scientific) at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. One week-old cultures will be used to re-inoculate 500 ml Erlenmeyer flasks containing 100 ml liquid BG11, which will then be incubated at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with a 24h lighting set. Heterotrophic cultures will be supplemented with 100 g L⁻¹ glucose. Samples will be collected at regular intervals and analyzed for linalool production, as well as chlorophyll content.

[00101] Chlorophyll will be measured with a spectrophotometer. To measure volatile linalool, 2 ml culture samples will be placed in sealed 20 ml headspace tubes, and incubated at 30°C for 2 hour. Volatiles will be sampled with a headspace sampler and measured by GC-MS. Linalool will be identified by comparison with genuine standard from GC-Standard grade liquid linalool. Linalool emission rates will be calculated in nmol g⁻¹ chlorophyll h⁻¹ over 2 hour incubation by headspace analysis. Linalool in the culture fluid will be measured by HPLC.

Results will be used to guide further genetic manipulations. FIG. 6. demonstrates the production of linalool in transgenic *Anabaena*.

[00102] b. Methylbutenol production

[00103] To engineer *Anabaena* to produce methylbutenol (MBO), methylbutenol synthase (MboS) will be transferred into *Anabaena*. The coding region of the MboS, with N-terminal plastid targeted sequence deletion, was cloned immediately downstream of the engineered translation initiation sequence (Shine-Dalargno sequence) under a dual promoter (*Pnir/PsbA*) in shuttle vector pZR807, a pNIR derived plasmid that replicates in *Anabaena*. Each construct was introduced into *Anabaena* by conjugation. Genetically engineered MBO-producing *Anabaena* strains (see above) will be grown in a liquid Bg11 medium which contains combined nitrogen in a lighted shaker (Innova 44R, New Brunswick Scientific) at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. One week-old cultures will be used to re-inoculate 4-liter Erlenmeyer flasks containing 1000 ml liquid BG11, which will then be incubated at 30°C and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ with a 24h lighting set. Samples will be collected at regular intervals (24h) and analyzed for MBO production. MBO excreted in the culture fluid will be measured by HPLC or GC/MS. Results will be used to guide further genetic manipulations.

[00104] c. Myrcene Production

[00105] To engineer *Anabaena* to produce myrcene, three MyrS genes in Table 2, i.e. ag2, ama0c15, and AtTPS10 will be transferred into the host. The coding region of the three genes, with N-terminal plastid targeted sequence deletion will be cloned immediately downstream of the engineered translation initiation sequence (Shine-Dalgarno sequence) under *Anabaena* psbA promoter (PpsbA) in shuttle vector pZR807, a plasmid that replicates in *Anabaena* and bears kanamycin resistance gene Kan^R. The constructs will be individually introduced into the host by conjugation. Genetically engineered *Anabaena* will be selected in a nitrate-containing AA/N medium agar plate supplemented with kanamycin sulfate. In certain experiments, a nitrate-inducible promoter will be used to replace the PpsbA promoter. In some experiments, an epitope tagged MyrS will be designed. The construct allows the 3' of MyrS gene in frame to link to FLAG₂-His₆ epitope tag engineered into the pZR807 vector once the MyrS gene stop codon is

removed. Genetically engineered myrcene-producing *Anabaena* strains will be grown as described for linalool-producing strain. The myrcene production will be measured by GC/MS as described for linalool measurement.

[00106] d. Farnesene Production

[00107] FPPS and FarS genes from *Artemisia* will be constructed as an operon under the control of the psbA promoter in shuttle vector pZR807. The construct will be individually introduced into *Anabaena* by conjugation. Genetically engineered *Anabaena* will be selected in a nitrate-containing AA/N medium agar plate supplemented with kanamycin sulfate. In certain embodiments, a nitrate-inducible promoter will be used to replace the PpsbA promoter. In some embodiments, an epitope tagged FarS will be designed. The construct allows the 3' of FarS gene in frame to link to FLAG₂-His₆ epitope tag engineered into the pZR807 vector once the FarS gene stop codon is removed. Farnesene produced by engineered *Anabaena* will be measured as described for linalool measurement.

[00108] EXAMPLE 7.

[00109] Optimization of Production of Carbon Based Products of Interest

[00110] a. RuBisCo/RuBisCo activase

[00111] The native RuBisCo genes rbcL/S (slr009/slr0012) and the putative RuBisCo activase (slr0011) gene will be over-expressed in hosts producing the carbon based product of interest. These three genes will be PCR amplified and fused to a strong *Anabaena* promoter PpsbA and subcloned into a shuttle vector for conjugation.

[00112] FBP/SBPase will be over-expressed to boost RUBP levels. Hosts producing carbon based products of interest will be genetically engineered with FBP/SBPase from *Synechococcus* PCC794. See Miyagawa Y, Tamoi M, Shigeoka S. 2001. Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. *Nat. Biotechnol.* 19(10):965-9 and Tamoi M, Nagaoka M, Miyagawa Y, Shigeoka S. 2006. Contribution of fructose-1,6-bisphosphatase and sedoheptulose-1,7-

bisphosphatase to the photosynthetic rate and carbon flow in the Calvin cycle in transgenic plants. *Plant Cell Physiol.* 47(3):380-90

[00113] b. ADP-GPPase

[00114] ADP-GPPase will be inactivated or deleted in certain genetically engineered *Anabaena*. ADP-GPPase may be inactivated using a double crossover knockout approach. This approach is well documented in Zhou R and Wolk CP. 2002 Identification of an akinete marker gene in *Anabaena variabilis*. *J. Bacteriol.* 184:2529-32 and Zhou R and Wolk CP 2003 A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J. Biol. Chem.* 278:19939-46. In *Anabaena*, the ADP-GPPase gene is all4645. As shown in FIG. 12, for example, LinS gene fused to *Anabaena* promoter is subcloned to an integration vector (fragment A and B are from *Anabaena* chromosome) and this resulting construction pLinS is then introduced to *Anabaena* for double homologous recombination at loci A and B of *Anabaena* chromosome. The double recombinants will be selected on the sucrose/Km plate by losing the conditional lethal gene *sacB* in the vector portion (Cai YP, Wolk CP. 1990. Use of a conditionally lethal gene in *Anabaena* sp. strain PCC 7120 to select for double recombinants and to entrap insertion sequences. *J. Bacteriol.* Jun; 172(6):3138–3145). The completely segregated double recombinants will be further verified by diagnostic PCR. Thus, the LinS/Km cassette from integration plasmid pLinS has replaced the gene all4645 (pink C in FIG. 12) in the double recombinants. In this example, gene all4645 has been deleted from *Anabaena* chromosome.

[00115] c. PDH

[00116] *Anabaena* PDH will be inactivated in some experiments. The internal fragment of *alr4745*, one of the three genes encoding *Anabaena* PDH multienzyme complex, will be amplified from *Anabaena* 7120 genomic DNA and cloned into pRL278, a plasmid designed for conjugative transfer into cyanobacteria. The *alr4745* will be knocked out according to the method disclosed in Zhou R and Wolk CP 2003 A two-component system mediates developmental regulation of biosynthesis of a heterocyst polysaccharide. *J. Biol. Chem.* 278:19939-46.

[00117] d. GGPPS/SQS

[00118] If a decrease in the FPP flux to terpenoids is desired, geranylgeranyl diphosphate synthase (GGPPS) and/or squalene synthase (SQS) expression will be down-regulated. SQS and or GGPS antisense sequences will be used to down-regulate GGPPS and/or SQS. The construct may additionally include an inducible promoter. The inducible promoter will be inducible by nitrate in many experiments. The gppS antisense sequence will be cloned downstream of a nitrate-inducible promoter and conjugatively transferred into hosts genetically engineered to produce target products. Down-regulating GPPS will be achieved by inducing antisense RNA expression with the addition of nitrate to the growth medium when cell density reaches the maximum.

[00119] e. FPPS

[00120] GPP flux will be optimized by down-regulating farnesyl-diphosphate synthase (FPPS). FPPS will be over-expressed in the antisense direction under an inducible promoter. The fppS antisense sequence will be cloned downstream of a nitrate-inducible promoter and conjugatively transferred into hosts genetically engineered to produce linalool or myrcene. Down-regulating FPPS is achieved by inducing antisense RNA expression with the addition of nitrate to the growth medium when cell density reaches the maximum.

[00121] f. Pyruvate synthesis

[00122] Pyruvate synthesis will be increased by over-expressing phosphoglycerate mutase, enolase, and pyruvate kinase (See FIG. 2). Three robust genes from *Z. mobilis* and from *S. cerevisiae* will be constructed as an artificial operon and fused to a PsbA1 promoter and then cloned into an integrative vector to insert the enzyme genes within the coding region of *alr4745* (encoding PDH-E3). This allows for increased synthesis of pyruvate while concurrently inactivating PDH.

[00123] GP3 flux may be altered by over-expressing certain rate-limiting enzymes. The DXS gene (*alr0599*) from *Anabaena* and the *Arabidopsis* IDI gene (AT5G16440) will be PCR

amplified with primers containing restriction sites and a ribosome binding site. The resulting PCR product will be fused to a nitrate-inducible promoter Pnir and cloned into pZR807.

[00124] All of the references cited herein are incorporated by reference in their entireties.

[00125] From the above discussion, one skilled in the art can ascertain the essential characteristics of the invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the embodiments to adapt to various uses and conditions. Thus, various modifications of the embodiments, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

SEQ ID NO. 1 Anabaena Pnir promoter sequence (590 bps):

GATCTAGCTACTCATTAGTTAAGTGTAATGCAGAAAACGCATATTCTCTATTAACT
TACGCATTAATACGAGAATTTTGTAGCTACTTATACTATTTTACCTGAGATCCCGACA
TAACCTTAGAAGTATCGAAATCGTTACATAAACATTCACACAAACCACTTGACAAAT
TTAGCCAATGTAAAAGACTACAGTTTCTCCCCGGTTTAGTTCTAGAGTTACCTTCAGT
GAAACATCGGCGGCGTGTCAGTCATTGAAGTAGCATAAATCAATTCAAAATACCCT
GCGGGAAGGCTGCGCCAACAAAATTAAATATTTGGTTTTTCACTATTAGAGCATCGA
TTCATTAATCAAAAACCTTACCCCCCAGCCCCCTTCCCTTGTAGGGAAGTGGGAGCC
AAACTCCCCTCTCCGCGTCGGAGCGAAAAGTCTGAGCGGAGGTTTCCTCCGAACAG
AACTTTTAAAGAGAGAGGGGTTGGGGGAGAGGTTCTTTCAAGATTACTAAATTGCTA
TCACTAGACCTCGTAGAACTAGCAAAGACTACGGGTGGATTGATCTTGAGCAAAAA
AACTTTATGAGAACGAATTTCG

SEQ ID NO. 2 Anabaena psbA1 promoter sequence (161 bp):

GGATTCCCAAAGATAGGGGGAATAATTAACATTAAGAATTATTAATTCATGGGTTTT
TAGTCTAGTAAATTTGCGTGAATTCATGTAAATTTTATGAGACAGGCGCAAGTCTAA
AAAAAGCGTCTGAATTAATCTGCACAAATCCAAAGCAATCATAAAAA

SEQ ID NO. 3 Anabaena PrbcL promoter sequence (402 bp):

GTTAACAAAACGTTTAAAACCTTTATGTAATAACAAATTTAAATATGTAAGTTAAGAA
 CTTTCAAAGAATAACTTATGCCATTTCTTGATATATTGTGAGACAAGTTACAAATTA
 CGTGGTGTGCAATTTTTTTCATCTTGCGCTGATTACTCTACTAAATATCCGTCAAGTAA
 ATTGGCTCTTAGCTCGTCTCCTGTCAATAAAGGAGGTCGGCAAGAGTGCAGAAGCG
 GGAATGTGTGAAAACCTAACCCAATTCATTAAATACCCCGAAATATAGGGGAATCAT
 CTCATACTTTCCGTAAACCGCGAAGGTCGTGAAGGGATAAAAGCAATTTAGTGGGT
 GAGAAGAACAGATAAAAAAGAATTTTTTAACTATGGCAAGAGGAAAAAGTAAAAG
 CGTTAAC

SEQ ID NO. 4 E. coli tac promoter sequence (244 bp):

CGACTGCACGGTGACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATG
 GCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTC
 TGGATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGAG
 CTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAAT
 AGGAAACAG

SEQ ID NO. 5 Pyruvate Decarboxylase [*Zymomonas mobilis* subsp. *mobilis* CP4]:

MSYTVGTYLAERLVQIGLKHHFAVAGDYNLVLLDNLLLKNMEQVYCCNELNCGFSA
 EGYARAKGAAAAVVTYSVGALSAFDAIGGAYAENLPVILISGAPNNNDHAAGHVLHHA
 LGKTDYHYQLEMAKNITAAAEAIYTPPEAPAKIDHVIKTALREKKPVYLEIACNIASMP
 AAPGPASALFNDEASDEASLNAAVEETLKFIANRDKVAVLVGSKLRAAGAEAAVKFA
 DALGGAVATMAAAKSFFPEENPHYIGTSWGEVSYPGVEKTMKEADAVIALAPVFNDYS
 TTGWTDIPDPKKLVLAEPRSVVVNGVRFPSVHLKDYLTRLAQKVSCKTGALDFFKSLN
 AGELKKAAPADPSAPLVNAEIARQVEALLTPNTTVIAETGDSWFNAQRMKLPNGARVE
 YEMQWGHIGWSVPAAFGYAVGAPERRNILMVGDSFQLTAQEVAQMVRCLKLPVIIFLI
 NNYGYTIEVMIHDGPYNNIKNWDYAGLMEVFNGNGGYDSGAGKGLKAKTGGELAEAI
 KVALANTDGPTLIECFIGREDCTEELVKWKGKRVAAANSRKPVNKLL

SEQ ID NO. 6 Alcohol Dehydrogenase II [*Zymomonas mobilis* CP4]:

MASSTFYIPFVNEMGEGSLEKAIKDLNGSGFKNALIVSDAFMNKSGVVKQVADLLKAQ
 GINSAVYDGVMPNPTVTAVLEGLKILKDNNSDFVISLGGGSPHDCAKAIALVATNGGEV

KDYEGIDKSKKPALPLMSINTTAGTASEMTRFCIITDEVHRHVKMAIVDRHVTMPMVSVD
 PLLMVGMPKGLTAATGMDALTHAFEAYSSTAATPITDACALKAASMIAKNLKTACDNG
 KDMPAREAMAYAQFLAGMAFNNASLGYVHAMAHQLGGYYNLPHGVCNAVLLPHVL
 AYNASVVAGRLKDVGVAMGLDIANLGDKEGAEATIQA VRDLAASIGIPANLTELGAKK
 EDVPLLADHALKDACALTNP RQGDQKEVEELFLSAF

SEQ ID NO. 7 >gil1488568|embl|CAA57500.1|sugar beet sucrose-phosphate synthase (SPS):
 MAGNDWINSYLEAILDVGPGLDDAKSSLLLRERGRFSPTRYFVEEVITGFDETDLHRWS
 VRAQATRSPQERNTRLENMCWRIWNLARQKKQLENEEAQRKTKRRMELERGRREATA
 DMSEDLSEGEKDISAHGDSTRPRLPRINSLDAMETWISQQKEKKLYLVLSLHGLIRGEN
 MELGRSDTGGQVKYVVELARALGSMGPVYRVDLLTRQVSSPDVDWSYGEPTMLNP
 RDSNGFDDDDDEMGESSGAYIVRIPFGPRDKYIAKEELWPYIPEFVDGALNHIVQMSKV
 LGEQIGSGETVWPVAIHGHYADAGDSAALLSGGLNVPMLLTGHSLGRDKLEQLLKQGR
 MSKDDINNTYKIMRRIEAEELSLDASEIVITSTRQEIEEQWHL YDGFDPVLERKLRARMK
 RGVSCYGRFMPRMVVIPPGMEFNHIVPHEGDM DGETEETEEHPTSPDPPIWAEIMRFFSK
 PRKPMILALARPDPKKNITTLVKAFGECRPLRELANLTLIMGNRDGIDEMSSTSSSVLLSV
 LKLIDQYDLYGQVAYPKHHKQADVPEIYRLAAKTKGVFINPAFIEPFGLTLIEAAAHGLP
 MVATKNGGPVDIQRVLDNGLLVPHEQQSIATALLKL VADKQLWTKCQQNGLKNIHL
 YSWPEHSKTYLSRIASSRQRQPQWQRSSDEGLDNQEPESPSDSL RDIKDISLNLEVLVRPE
 KRVKTLKILGLMTKANSRMLLCWSNGVHKMLRKARFSDKVDQASSKYPAFRRRKLIY
 VIAVDGDYEDGLFDIVRRIFDAAGKEKIEGSIGFILSTSYSMPEIQNYLLSKGFNLHDFDA
 YICNSGSELYYSSLNSEESNIIADSDYHSHIEYRWGGEGLRRTLLRWAASITEKNGENEEQ
 VITEDEEVSTGYCFAFKIKNQNKVPPTKELRKSMRIQALRCHVIYCQNGSKMNVIPVLAS
 RSQALRYLYVRWGVLSKMOVVFVGECDTDYEGLLGGVHKT VILKGVSN TALRS LHA
 NRSYPLSHVVSLDSPNIGEVSKGCSSEIQSIVTKLSKA

SEQ ID NO. 8 >gil302826884|gbl|ADL70859.1| Sugarcane sucrose phosphate synthase (SPS):
 MAGNDWINSYLEAILDAGGAAGEISAAAGSGGGGDGTAGEKRDKSSMLLRERGRFNPA
 RYFVEEVISGFDETDLYKTWVRTSAMRSPQERNTRLENMSWRIWNLARKKKQIKGEEA
 SRLSKRRMELEKARQYAATDLS EDLSEGEKGETNNEPSIHDESMRTRMPRIGSTDAIET
 WANQHKDKKLYIVLISIHGLIRGENMELGRSDTGGQVKYVVELARALGSTPGVYRVD

LLTRQISAPDVDWSYGEPTMLSPISSENFGEHELGESSGAYIVRIPFGPRDKYIPKEHLWP
 HIQEFVDGALVHIMQMSKVLGEQIGSGQPVPVVIHGHYADAGDSAALLSGALNVPMV
 FTGHS�GRDKLEQILKQGRQTRDEINATYKIMRRIEAEELCLDTSEIITSTRQEIEQQWGL
 YDGFDLTMARKLRARIKRGVSCFGRYMPRMIAIPPGMEFSHIAPHDVDLDSEEGNEDGS
 GSPDPPIWADIMRFFSNPRKPMILALARPDPKKNITTLVKAFGEHRELRLANLTLIMGN
 RDVIDEMSSTNAAVLTSVLKLIDKYDLYGQVAYPKHHKQFEVPDIYRLAARTKGVFINC
 AFIEPFGLTLIEAAAYGLPIVATRNGGPVDIHRVLDNGILVDPHNQNEIGEALYKLVSDKQ
 LWTRCRQNGLKNIHQFSWPEHCKNYLARVVTLKPRHPRWQKNDVATEISEADSPEDSL
 RDIHDISLNLQLSLDSEKSGSKEGNSNTVRRHLEDVQKLSGVSDIKKDGPGENGKWPS
 LRRRKHIIVIAVDSVQDADFVQVIKNIFEASSNERSSGAVGVFLSTARAISEIHALLISGRIE
 ASDFDAFICNSGSDLCYPSSSEDMLSPAELPFMIDLDYHSQIEYRWGGEGLRKTLIRWA
 AEKNNESGQKILVEDEECSTYCSFKVSNTAAAPPVKEIRRTMRIQALRCHVLYSHDGS
 KLVNIPVLASRSQALRYLYIRWGVELSNTVIVGECGDTDYEGLLGGVHKTIILKGSFNT
 APNQVHANRSYSLQDVVSFEKQGISSIEGYGPDNLKSALRQFGILKD

SEQ ID NO. 9 >gil69205229|gb|AAZ03743.1|Sugarcane sucrose phosphate phosphatase(SPP):
 MDKLSGSVRLMIVSDLDHTMVDHHDEENLSLLRFGALWESVYCEDSLLVFSTGRSPTL
 YKELRKEKPMLTPDITIMSVGTEITYGEAMVPDDGWEQYLNNKWDRNIVVEETASFSEL
 KLQPETEQRPBKVSFLVDKKSQAQEVKSVARLDKRGLDAKIIYSGGQDLDPQAGAGK
 GQALAYLLKKLSSCGKPPNNTLVCGDSGNDALFSIPGVMVSNAQEELLQWYAENAKD
 NPKIIHANERCAAGIIQAIGHFKLGPNISPRDVFYPYAKEASFKPSDAVVKFYVLYEKWR
 RAEVPSKSDSVIKYFKNITHANGVIIHPAGLELSLHASIDALGSCYGDQKQKGYRAWVDR
 LAITQTGSDSWVVRFDLWESEGDVRVCSLSSLALVLKAESPEGFVLTHIQKTWLNQYSS
 GVEQAFKV

SEQ ID NO. 10 Synechocystis sucrose phosphate synthase (SPS):
 MSYSSKYILLISVHGLIRGENLELGRDADTGGQTKYVLELALALVKNPQVARVDLLTRLI
 KDPKVDADYAQPRELIGDRAQIVRIECGPPEYIAKEMLWDYLDNFADHALDYLKEQPEL
 PDVIHSHYADAGYVGTRLSHQLGIPLVHTGHS�GRSKRTRLLLSGIKADEIESRYNMARR
 INAEETLGSAARVITSTHQEIAEQYAYDYYPQDQMLVIPPGTDLEKFYPPKGNEWETP
 IVQELQRFLRHPRKPIILALSRPDPRKNIHKLIAAYGQSPQLQAQANLVIVAGNRDDITDL

DQGPREVLTDLLLTIDRYDLYGKVA YPKQNAEDVYALFRLTALSQGVFINPALTEPFG
 LTLIEAAACGVPIVATEDGGPVDIKNQNGYLINPLDEVDIADKLLKVLNDKQQWQFLS
 ESGLEGVKRHYSWPSHVESYLEAINALTQQTSVLKRSCLKRRRTLYYNGALVTSLDQNL
 LGALQGGPLPGDRQTLDELLEVLYQHRKNVGFCIATGRRLDSVLKILREYRIPQPDMLITS
 MGTEIYSSPDLPDQSWRNHIDYLWNRNAIVRILGELPGLALQPKEELSAYKISYFYDAAI
 APNLEEIRQLLHKGEQTVNTIISFGQFLDILPIRASKGYAVRWLSQQWNIPLEHVFTAGGS
 GADEDMMRGNTLSVVVANRHHEELSNLGEIEPIYFSEKRYAAGILDGLAHYRFFELLDP
 V

SEQ ID NO. 11 Synechocystis sucrose-phosphate phosphatase (SPP)

MRQLLLISDLNTWVGDDQQALEHLQEYLGDRRGNFYLAYATGRSYHSARELQKQVGL
 MEPDYWLTA VGSEIYHPEGLDQHWADYLSEHWQRDILQAIADGFEALKPQSPLEQNPW
 KISYHLDPQACPTVIDQLTEMLKETGIPVQVIFSSGKDVDLLPQRSNKG NATQYLQQHLA
 MEPSQTLVCGDSGNDIGLFETSARGVIVRNAQPELLHWYDQWGDSRHYRAQSSHAGAI
 LEAIAHFDFLS

SEQ ID NO. 12 ZmSUT1: accession AB008464.1:

MARGDGELELSVGVRGTGGAAAAAADHVAPISLGRLLLAGMVAGGVQYGWALQLSL
 LTPYVQTLGLSHALT SFMWLCGPIAGLVVQPLVGLYSDRCTARWGRRRPFILIGCMLICL
 AVIVVGFSSDIGAALGDTKEHCSLYHGPRWHAAIVYVLGFWLLDFSNNTVQGPARAMM
 ADLCGHHGPSAANSIFCSWMALGNILGYSSGSTNNWHKWFPFLLTNACCEACANLKGA
 FLVAVVFLVMCLTVTLFFANEVPYRGNQNLPTKANGEVETEP SGPLAVLKGFKNLPTG
 MPSVLLVTGLTWLSWFPFILYDTDWMGREIYHGDPKGSNAQISAFDEGVRVGSFGLLLN
 SIVLGFSSFLIEPMCRKVGPRVWVTSNFMVCVAMAATALISFWSLKDYHGYVQDAITA
 STSIKAVCLVLFAFLGVPLAILYSVPFVTAQLAATKGGGQGLCTGVLNISIVIPQVIALG
 AGPWDALFGKGNIPAFGVASGFALIGGVGVFLLPKISKRQFRAVSAGGH

SEQ ID NO. 13 AAV41028.1| sucrose transporter [Saccharum hybrid cultivar Q117]:

MARGDGELELSVGVRGAGAAAADHVAPISLGRLLLAGMVAGGVQYGWALQLSLLTPY
 VQTLGLSHALT SFMWLCGPIAGLVVQPLVGLYSDRCTARWGRRRPFILTGILISLAVIV
 VGFSSDIGAALGDTKEHCSLYHGPRWHAAIVYVLGFWLLDFSNNTVQGPARAMMADL

CGHHGPSAANSIFCSWMALGNILGYSSGSTNNWHKWFPFLKTNACCEACANLKGAFLV
AVVFLVICLAVTLIFAKEVPYRGNNENLPTKANGEVEAEPTGPLAVLKGFKNLPPGMPSV
LLVTGLTWLSWFPFILYDTDWMGREIYHGDPKGSNAQISAFNEGVRVGAFGLLLSIILG
FSSFLIEPMCRKLGPRVWVTSNFMVCVAMAATALISYWSLKDYHGYVQDAITASTNIK
AVCLVLFAFLGVPLAILYSVPFAVTAQLAATKGGGQGLCTGVLNISIVIPQVIIALGAGPW
DALFGKGNIPAFGVASGFALIGGVVGVFLLPKISKRQFRAVSAGGH

SEQ ID NO. 14 *Anabaena cylindrica* acyl-ACP reductase (AR) coding sequence:

ATGCAGCAGCTTGTGAGCAAATTGAAAAAATTGATTTCCAAAGTGAAGAATACAA
AGACGCATATAGCCGTATTAATGCAATTGTGATTGAAGGGGAACAAGAAGCCCATG
ATAATTACATTCAACTGGCGGAAGTCTGCCAGAAAGTAAAGACAACCTGATTCGCT
TATCGAAGATGGAAAGCCGTCACAAGAAAGGATTTGAAGCTTGTGGACGCAATTTG
CAGGTCACACCAGACATGAAGTTTGCAAAGAGTTTTTCTCAGGACTGCACAAAAA
TTTTCAAAGTGGCGCCGCAGAAGGTAAAGTTGTTACTTGCTTGCTGATTCAAGCTTT
AATTATCGAATGTTTTGCGATCGCAGCATACAACATCTACATTCCCGTCGCTGATGA
TTTCGCCCCGAAAATTACAGAAGGTGTGGTCAAAGAAGAATACAGTCATCTCAATTT
TGGCGAAGTTTGGCTTCAAGAAAAGTTTGCAGAATCCAAAGCTGAATTAGAAACAG
CTAACCGCCAAAATCTTCCCCTAGTCTGGAAGATGCTCAACCAAGTAGCAGATGATG
CCCACGTCTTGGCAATGGAAAAAGAAGCCTTAGTAGAAGATTTTCATGATTCAATACG
GTGAGGCACTAAGTAATATTGGCTTCACAAGTCTGATATTATGCGTCTCTCCGCTT
ACGGACTCATACCTGTCTAA

SEQ ID NO. 15 *Anabaena cylindrica* aldehyde decarboxylase (AD) coding sequence:

ATGTTTGGTCTAATTGGACATCTGACTAGCTTAGAACACGCTCAATCCGTAGCTCAA
GAATTGGGATACCCAGAATATGCCGATCAAGGGCTAGACTTTTGGTGTAGCGCCCCG
CCGCAAATTGTCGATCACATTACCGTTACCAGCATCACCGGACAAAAAATTGAAGGT
CGGTATGTAGAATCTTGCTTTTTGCGGAAATGCTGGCAAATCGCCGCATTAAAGCT
GCAACTCGCAAATCTCAACGCCATGGCTCATGCTCAAAGCATGGCATTGATATC
ACGGCTTTAGGTGGGTTTTCTTCAATTATTTTGAGAACTTCAATTTAGAGCAGTTTA
GCCAAGTCCGAAACGTAAATTAGAATTTGAACGCTTCACAACAGGAAATACCCAT
ACAGCCTACATCATCTGTCGGCAGGTAGAGGAAGCATCTAAGCAATTAGGAATAGA

ATTGTCAAAAGCAACTGTGGCTGTGTGTGGCGCTACAGGGGATATTGGCAGTGCAGT
TACCCGCTGGTTAGATAAAAAACAGATGTCCAAGAATTACTCCTCATAGCCCGTAA
CCAAGAACGTCTTCAAGAACTACAAGCAGAATTGGGACGGGGTAAAATCATGGGTT
TACAGGAAGCATTACCCCAAGCCGATATTGTAGTTTGGGTTGCTAGTATGCCTAAAG
GTGTAGAAATTGACCCCAACCGTACTGAAACAACCTTGTTTGCTGATTGATGGTGGCT
ATCCTAAAACTTAGGGACAAAAATTCAGCATCCTGGCGTGTATGTATTAAATGGTG
GAATTGTGAGCATTCCCTAGATATTGACTGGAAAATTATGAAAATTGTCAATATGG
ATGTCCCAGCACGCCAGTTGTTTGCTTGTTTTGCGGAATCAATGCTGCTGGAATTTGA
GAAGTTATACACAACTTTTCTTGGGGTCGTAATCAGATTACCGTAGATAAAATGGA
GCAAATTGGTTCGGGTGTCAATTAAACACGGTTTTAGACCATTATTAGTTTAG

SEQ ID NO. 16 MboS accession # JF719039:

ATGGCTCTGCTCTCTGTCGCACCGCTGGCTCCCAGATGGTGCGTGCACAAATCGTTG
GTCACTTCTACCAAGGTAAAGGTTGTCCGCAGAACGATCTCAACTCCATCCGCATG
TGTCGGATAACCACTGAATCCGGTGAAGGCGTACAGAGACGCATAGCAAATCATCA
TTCCAACCTCTGGGACGATAATTTCATACAGTCCCTCTCAACGCCTTATGGGGCAAT
TTCGTACCATGAAAGTGCTCAGAACTTATTGGAGAAGTAAAAGAGATGATCAATT
CAATCTCGCTTAAAGATGGAGAATTAATCACCCCTCCAATGATCTCCTTATGCGGC
TCTCTATAGTCGATAGCATTGAACGTTTGGGAATCGATAGGCATTTCAAAAGTGAAA
TAAAATCAGCTCTGGATTATGTTTACAGTTATTGGAACGAAAAAGGCATTGGGTGGG
GAAGAGATAGTGTTGTTGCCGATCTCAACTCAACTGCCTTGGGGCTTCGAACTCTAC
GACTACACGGATACCCGGTGTCTTCAGATGTGTTACAACACTTCAAAGAACAAAA
GGGCAGTTTGCATGTTTCGGCCATTCAAACAGAGGGAGAGATAAGAAGTGTTCTCAA
CTTATTTTCGGGCTTCCCAAATTGCCTTTCCGGGAGAGAAAGTTATGGAAGAGGCAGA
AGTCTTCTCTACAATATATTTAAAAGAAGCCATACTAAAGCTTCCGGTCTGCGGTCT
TTCACGAGAGATATCGTACGTTCTGGAATATGGTTGGCATATAAATTTGCCAAGATT
GGAAGCAAGGAACTACATCGACGTATTTGGAGAGGACCCCATTTATTTGACGCCAA
ATATGAAGACCCAAAACTTCTAGAACTTGCAAAGTTGGAGTTCAATATGTTTCACT
CTTTACAACAGCAAGAGCTAAAGCTTCTCTCCAGATGGTGGAAAGATTCGGGTTTCT
CTCAAATGACCTTCCCTCGGCATCGTCACGTGGAATATTACACTTTGGCATCTTGCAT
TGATAGTGAACCTCAACATTCTTCGTTTCAGACTTGGATTTGCCAAAATCTTTCATCTT

GCCACGGTTCTTGACGATATTTACGACACCTTTGGCACGATGGATGAGCTAGAACTC
TTCACGGCGGCAGTTAAGAGGTGGCATCCGTCTGCGACGGAGTGGCTTCCAGAATAT
ATGAAAGGAGTATATATGGTGCTTTACGAAACCGTTAACGAAATGGCAGGAGAAGC
AGAAAAGTCTCAAGGCCGAGACACGCTCAACTATGGCCGAAATGCTTTGGAGGCTT
ATATTGATGCTTCTATGGAAGAAGCGAAGTGGATTTTCAGTGGTTTTTTGCCAACAT
TTGAGGAGTACCTGGATAACGGGAAAGTTAGTTTCGGTTATGGCATTGGCACATTGC
AACCATTCTGACGTTGGGCATTCCCTTTCCTCATCACATCCTACAAGAAATAGACTT
TCCTTCCAGGCTCAATGATGTGGCATCTTCCATTCTCCGACTAAAAGGCGACATTCA
CACTTACCAGGCTGAGAGGAGCCGTGGAGAAAAATCTTCGTGTATATCATGTTATAT
GGAAGAGAATCCCGAGTCAACAGAGGAAGATGCAATCAATCATATCAACTCCATGG
TCGACAAATTACTCAAGGAACTAAATTGGGAGTATCTGAGACCTGATAGCAATGTTC
CAATCACTTCCAAGAAACATGCATTTGACATTCTGAGAGCTTTCTACCATCTCTACA
AATACCGAGATGGCTTCAGCGTTGCGAACTATGAAATAAAGAATTTGGTCATGACA
ACCGTCATTGAGCCTGTGCCTTTATA

CLAIMS

What is claimed is:

1. A host genetically engineered with at least one recombinant polynucleotide expression construct, wherein the at least one recombinant polynucleotide expression construct comprises a nucleotide sequence encoding at least one enzyme, wherein the at least one enzyme increases production of at least one carbon based product of interest by the host following expression of the polynucleotide expression construct.
2. The composition of claim 1 wherein the host is an *Anabaena* spp.
3. The composition of claim 2 wherein the *Anabaena* spp. is *Anabaena* PCC7120.
4. The composition of claim 2 wherein the *Anabaena* spp. is *Anabaena cylindrica* 29414.
5. The composition of claim 2 wherein the *Anabaena* spp. is *Anabaena variabilis* ATCC29413.
6. The composition of claim 1 wherein the host has an inactive ADP-GPPase.
7. The composition of claim 1 wherein the host has an inactive PDH.
8. The composition of claim 1 wherein the host has a down-regulated GGPPS.
9. The composition of claim 1 wherein the host has a down-regulated FPPS.
10. The composition of claim 1 wherein the host has a down-regulated squalene synthase.
11. The composition of claim 1 wherein the host has increased pyruvate synthesis.

12. The composition of claim 11 wherein the pyruvate synthesis is increased by over-expressing phosphoglycerate mutase.
13. The composition of claim 11 wherein the pyruvate synthesis is increased by over-expressing enolase.
14. The composition of claim 11 wherein the pyruvate synthesis is increased by over-expressing pyruvate kinase.
15. The composition of claim 1 wherein the host has an up-regulated MEP pathway.
16. The composition of claim 15 wherein the up-regulated MEP pathway is up-regulated by expressing at least one gene responsible for control of the MEP pathway in the host.
17. The composition of claim 15 wherein the up-regulated MEP pathway is up-regulated by expressing all eight genes responsible for control of the MEP pathway in the host.
18. The composition of claims 1-5 and claims 11-14 wherein the at least one carbon based product of interest is ethanol.
19. The composition of claim 18 wherein the at least one enzyme is pyruvate decarboxylase (PDC).
20. The composition of claim 18 wherein the at least one enzyme is alcohol dehydrogenase (ADH).
21. The composition of claim 18 wherein the at least one recombinant polynucleotide expression construct further comprises a nucleotide sequence encoding RuBisCo.

22. The composition of claim 21 wherein the at least one recombinant polynucleotide expression construct comprising a nucleotide sequence encoding RuBisCo, further comprises a nucleotide sequence encoding RuBisCo activase.
23. The composition of claims 1-5 wherein the at least one carbon based product of interest is sucrose.
24. The composition of claim 23 wherein the at least one enzyme is sucrose phosphate synthase (SPS).
25. The composition of claim 23 wherein the at least one enzyme is sucrose phosphate phosphatase (SPP).
26. The composition of claim 23 comprising at least two enzymes, wherein the at least two enzymes are sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP).
27. The composition of claim 26 wherein the host over-expresses at least one sucrose exporter gene.
28. The composition of claim 27 wherein the at least one sucrose exporter gene is ZmSUTL.
29. The composition of claim 26 comprising at least three enzymes, wherein the at least three enzymes are sucrose phosphate synthase (SPS), sucrose phosphate phosphatase (SPP) and sucrose isomerase.
30. The composition of claim 23 wherein the host has at least one down-regulated sucrose degradation gene.
31. The composition of claim 29 wherein the down-regulated gene is a gene encoding invertase.

32. The composition of claim 30 wherein the down-regulated gene is a gene encoding sucrose synthase.
33. The composition of claim 30 wherein the down-regulated gene is a gene encoding phosphofructokinase.
34. The composition of claims 1-5 wherein the at least one carbon based product of interest is urea.
35. The composition of claim 34 wherein the at least one enzyme is arginase.
36. The composition of claim 35 wherein a PatA gene encodes a protein capable of regulating heterocyst spacing.
37. The composition of claim 34 wherein the host over-expresses at least one urea cycle gene.
38. The composition of claim 37 wherein the host over-expresses at least four urea cycle genes.
39. The composition of claim 38 wherein the over-expressed urea cycle genes are CPS-1, OTC, ASS, and AS.
40. The composition of claim 34 wherein the host has a down-regulated cyanophycin synthetase.
41. The composition of claims 1-5 and claims 15-17 wherein the at least one carbon based product of interest is a long chain alkane or a long chain alkene.
42. The composition of claim 41 wherein the at least one enzyme is acyl-ACP reductase (AR).

43. The composition of claim 41 wherein the at least one enzyme is aldehyde decarbonylase (AD).
44. The composition of claim 41 comprising at least two enzymes, wherein the at least two enzymes are acyl-ACP reductase (AR) and aldehyde decarbonylase (AD).
45. The composition of claims 1-5 and claims 15-17 wherein the at least one carbon based product of interest is methylbutenol.
46. The composition of claim 45 wherein the at least one enzyme is methylbutenol synthase.
47. The composition of claims 1-5 and claims 15-17 wherein the at least one carbon based product of interest is linalool.
48. The composition of claim 47 wherein the at least one enzyme is linalool synthase.
49. The composition of claims 1-5 and 15-17 wherein the at least one carbon based product of interest is myrcene.
50. The composition of claim 49 wherein the at least one enzyme is myrcene synthase.
51. The composition of claims 1-5 and 15-17 wherein the at least one carbon based product of interest is farnesene.
52. The composition of claim 51 comprising at least two enzymes, wherein the at least two enzymes are farnesene synthase and FPP synthase.
53. A method of genetically engineering a host to be capable of producing a carbon based product of interest comprising:

- (a) introducing a recombinant enzyme into the host, wherein the recombinant enzyme can participate in the host's natural metabolic pathway,
- (b) modifying at least one competing metabolic pathway to increase production of the carbon based product of interest.

54. The method of claim 53 wherein the host is *Anabaena* spp.

55. The method of claim 54 wherein the *Anabaena* spp. is the ethanol producing *Anabaena* sp. PCC7120 (pZR672) strain deposited under ATCC accession number XXX or the linolool producing *Anabaena* sp. PCC7120 (pZR808) strain deposited under ATCC accession number XXX.

56. The method of claim 54 wherein the host natural metabolic pathway is the MEP pathway.

57. The method of claim 54 wherein the host natural metabolic pathway is the Calvin Cycle.

58. An isolated *Anabaena* spp., wherein the *Anabaena* spp. is the ethanol producing *Anabaena* sp. PCC7120 (pZR672) strain deposited under ATCC accession number XXX.

59. An isolated *Anabaena* spp., wherein the *Anabaena* spp. is the linolool producing *Anabaena* sp. PCC7120 (pZR808) strain deposited under ATCC accession number XXX.

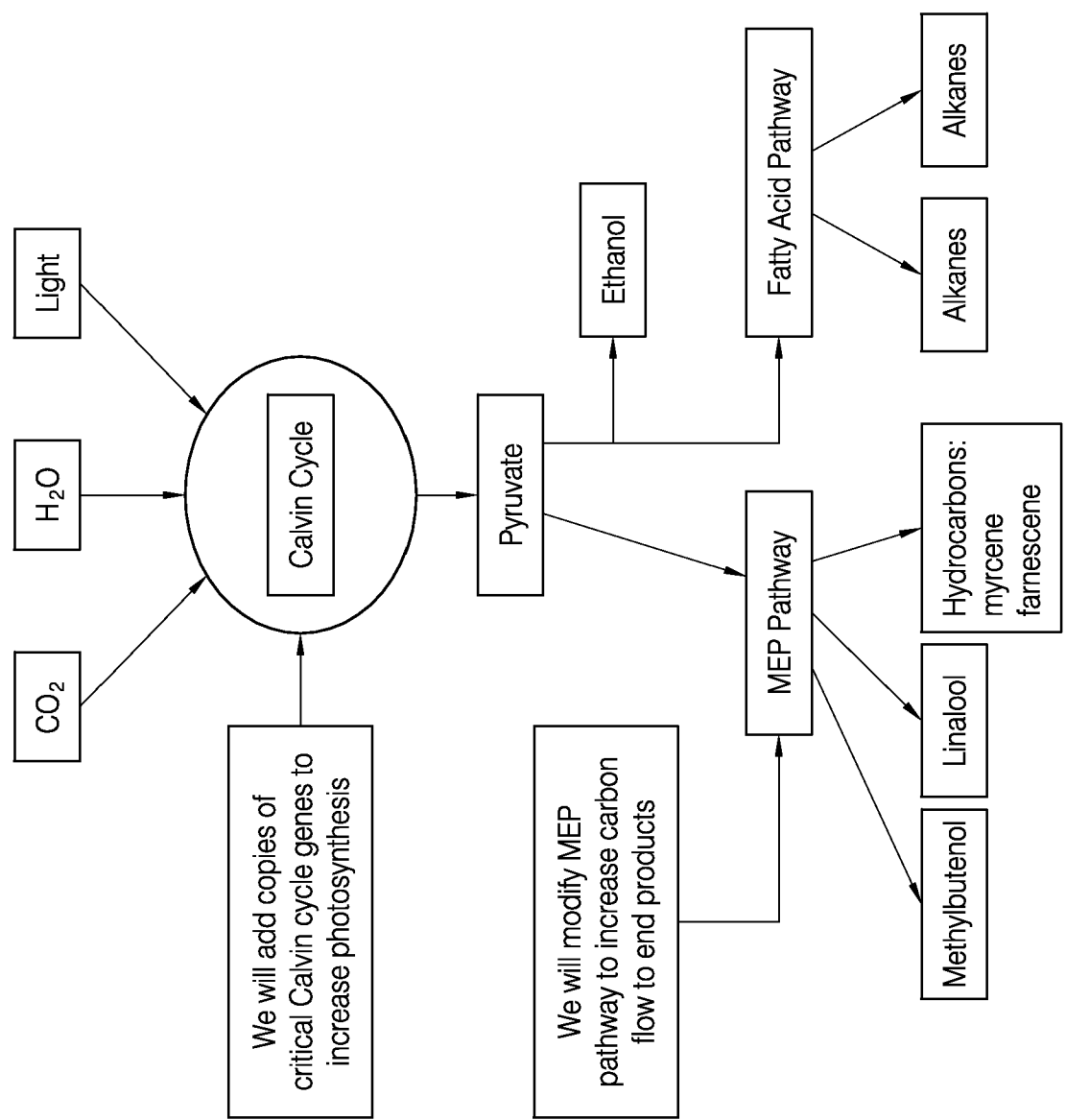
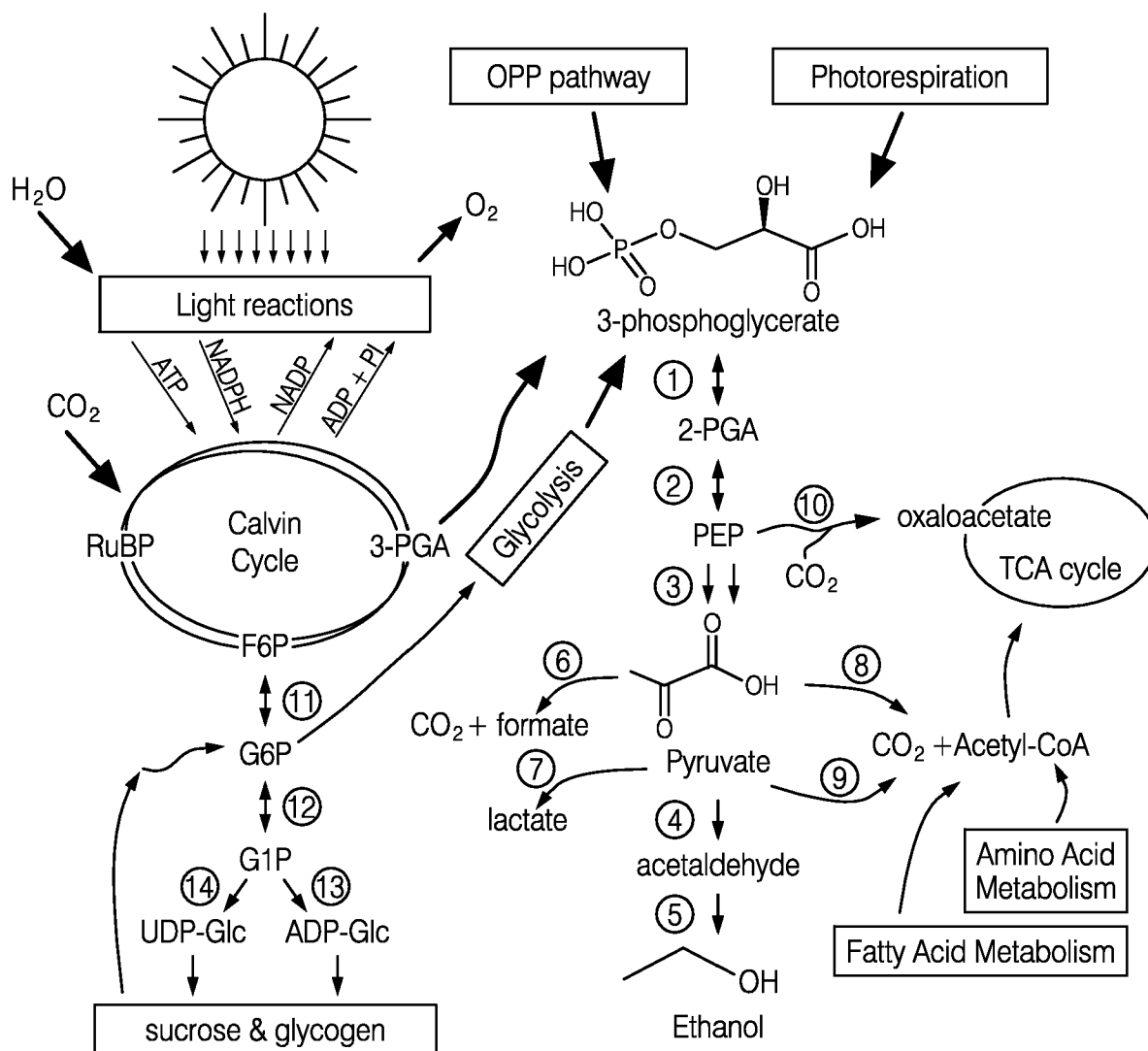


FIG. 1

2/15



- ① phosphoglycerate mutase
- ② enolase
- ③ pyruvate kinase
- ④ pyruvate decarboxylase
- ⑤ alcohol dehydrogenase
- ⑥ pyruvate formate lyase
- ⑦ lactate dehydrogenase

- ⑧ pyruvate: ferredoxin oxidoreductase
- ⑨ pyruvate dehydrogenase
- ⑩ phosphoenolpyruvate carboxylase
- ⑪ phosphoglucose isomerase
- ⑫ phosphoglucose mutase
- ⑬ ADP-glucose pyrophosphorylase
- ⑭ UDP-glucose pyrophosphorylase

The heavy green and red arrows indicate the carbon flow from CO₂ to ethanol in the engineered cyanobacterium.

FIG. 2

Our transgenic *Anabaena* eat CO₂ and sunlight
to produce and secrete ethanol! HPLC detected
current ethanol productivity is 13.8 mg/liter/h

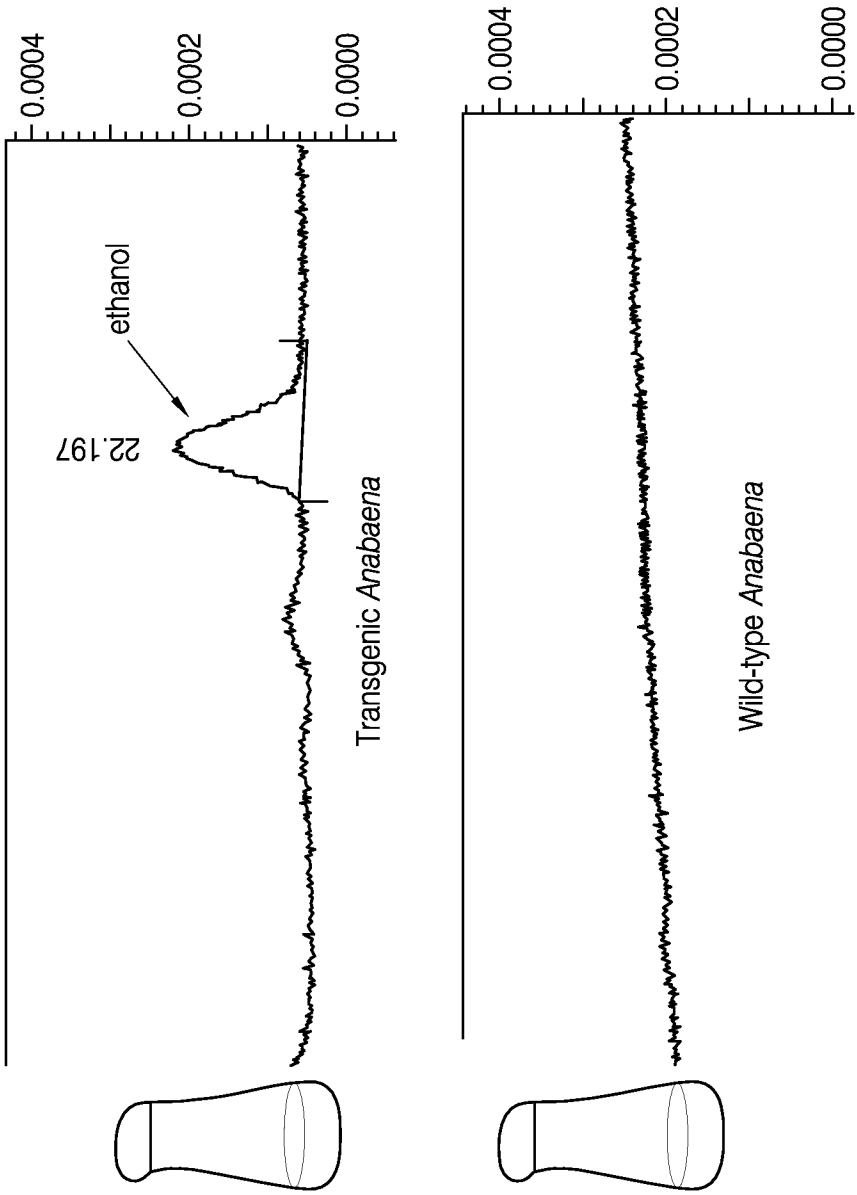
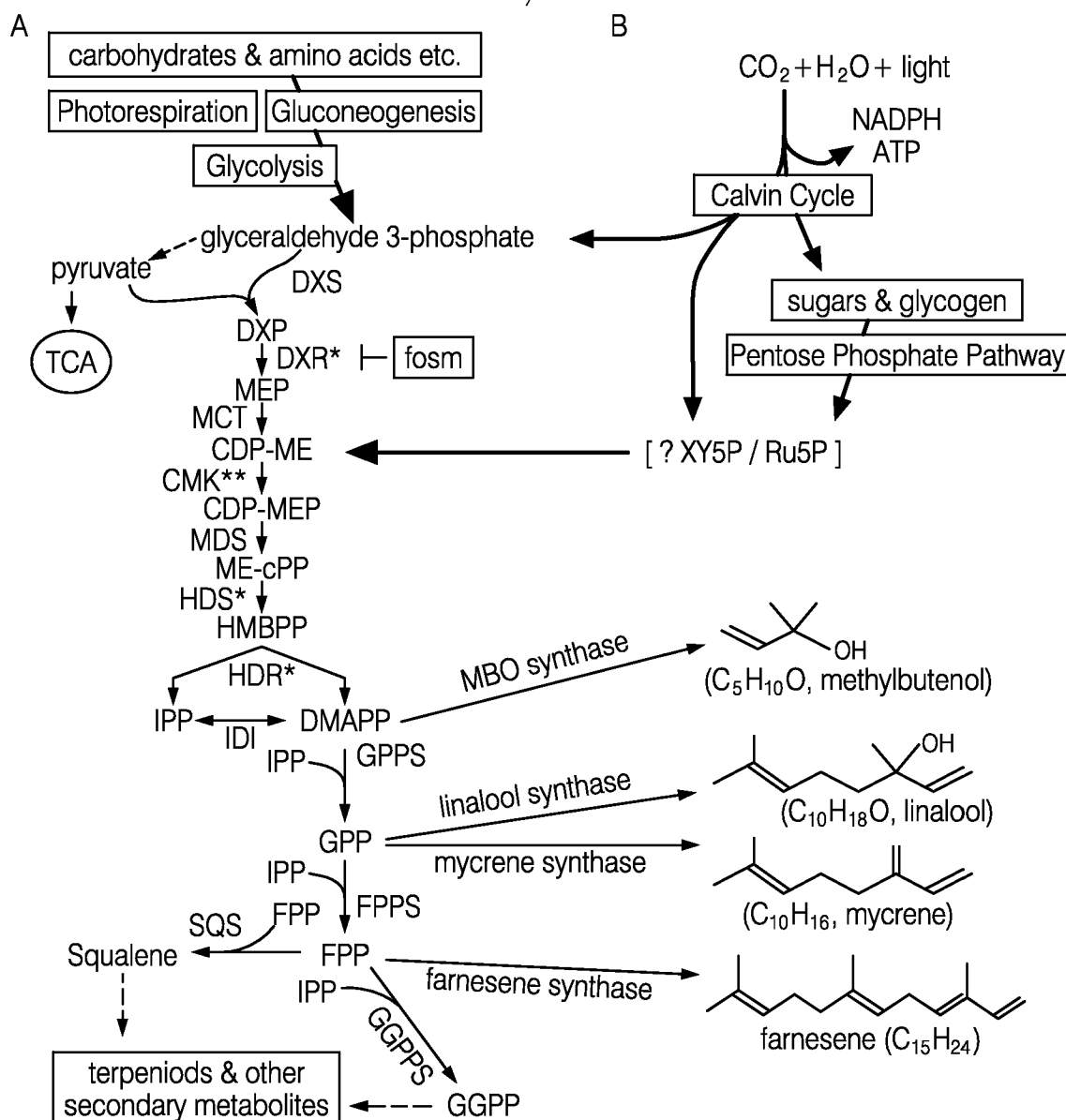


FIG. 3

4/15



Engineering cyanobacteria to produce lon-chain hydrocarbons

A. Known MEP pathway exists in plants, algae, and some bacteria;

B. Proposed a photosynthetic MEP pathway in cyanobacteria.

*NADPH as cofactor. **ATP dependent

G3P	D-glyceraldehyde-3-phosphate	Ru5P	ribulose-5-phosphate
DXP	1-deoxy-D-xylulose 5-phosphate	DXS	DXP synthase
MEP	methylerythritol-4-phosphate	DXR	DXP reductoisomerase
CDP-ME	diphosphocytidyl methylerythritol	MCT	CDP-ME synthase
CDP-MEP	CDP-methylerythritol-2-phosphate	CMK	CDP-ME kinase
ME-cPP	methylerythritol-2,4-cyclodiphosphate	MDS	ME-cPP synthase
HMBPP	hydroxymethylbutenyl diphosphate	HDS	HMBPP synthase
IPP	isopentenyl diphosphate	HDR	HMBPP reductase
DMAPP	dimethylallyl-diphosphate	IDI	IPP:DMAPP isomerase
GPP	geranyl-diphosphate	GPPS	GPP synthase
FPP	farnesyl-diphosphate	FPPS	FPP synthase
GGPP	geranylgeranyl-diphosphate	GGPPS	GGPP synthase
XY5P	xylulose-5-phosphate	SQS	squalene synthase

FIG. 4

5/15

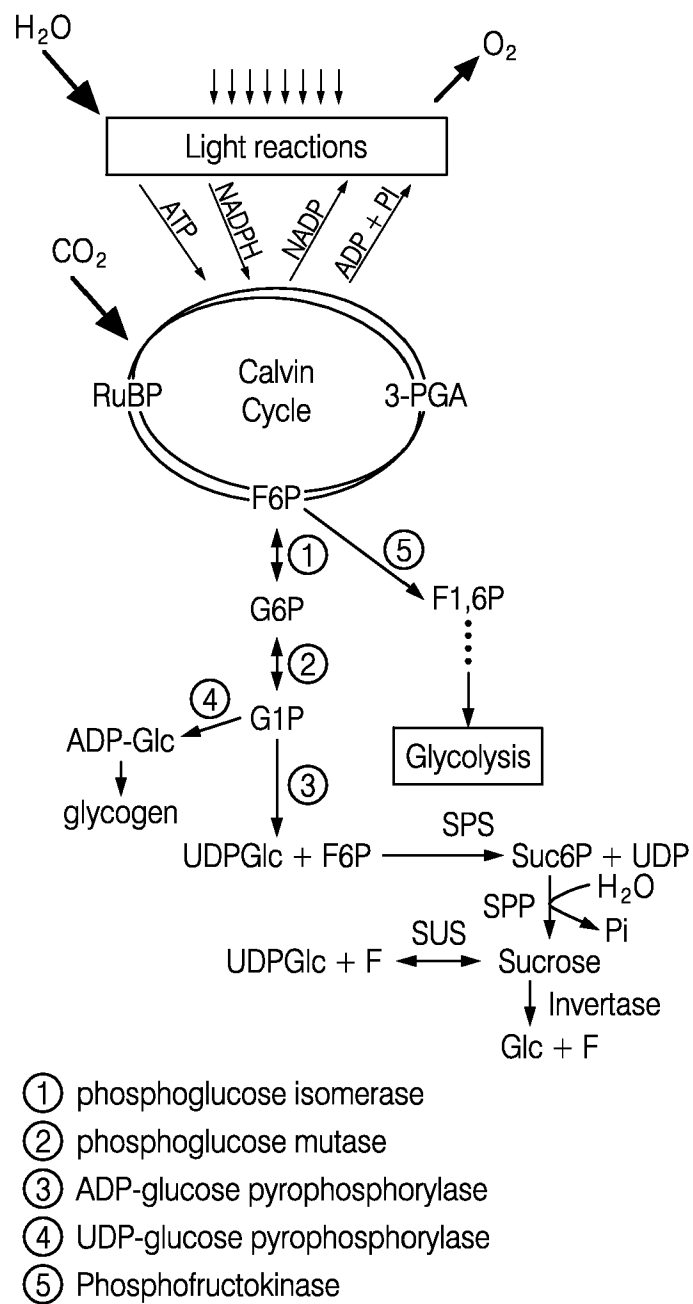


FIG. 5

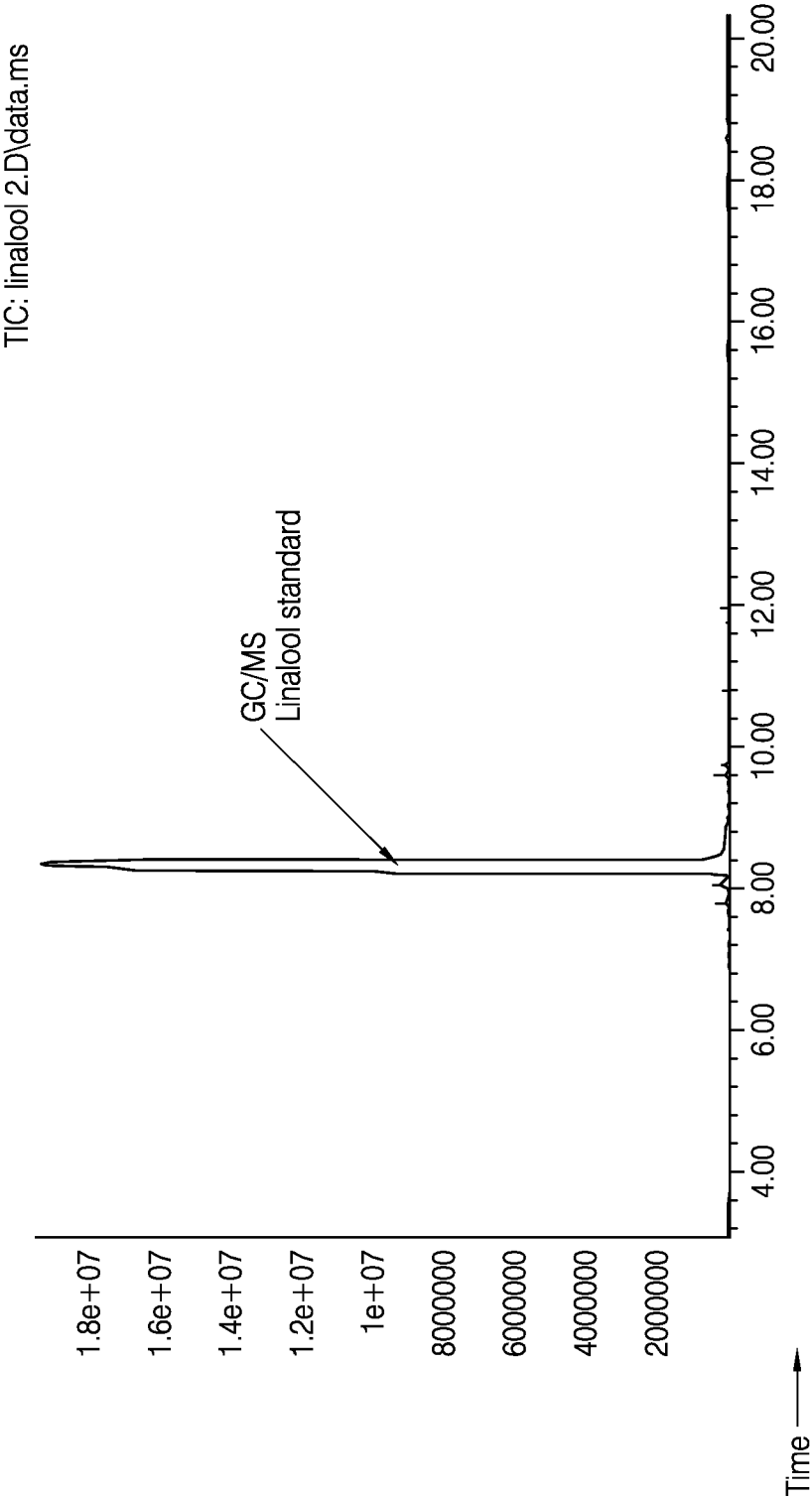


FIG. 6A

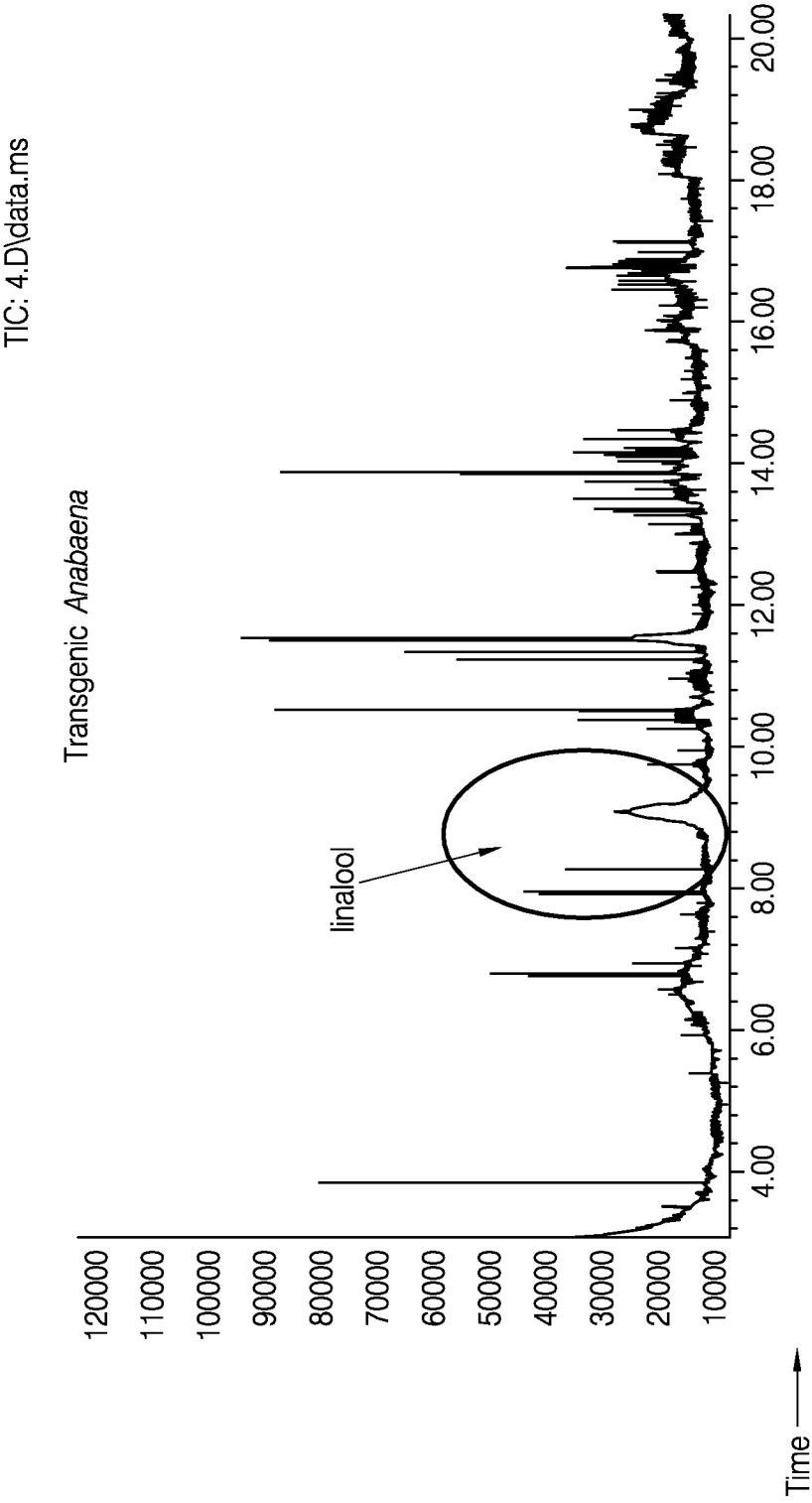


FIG. 6B

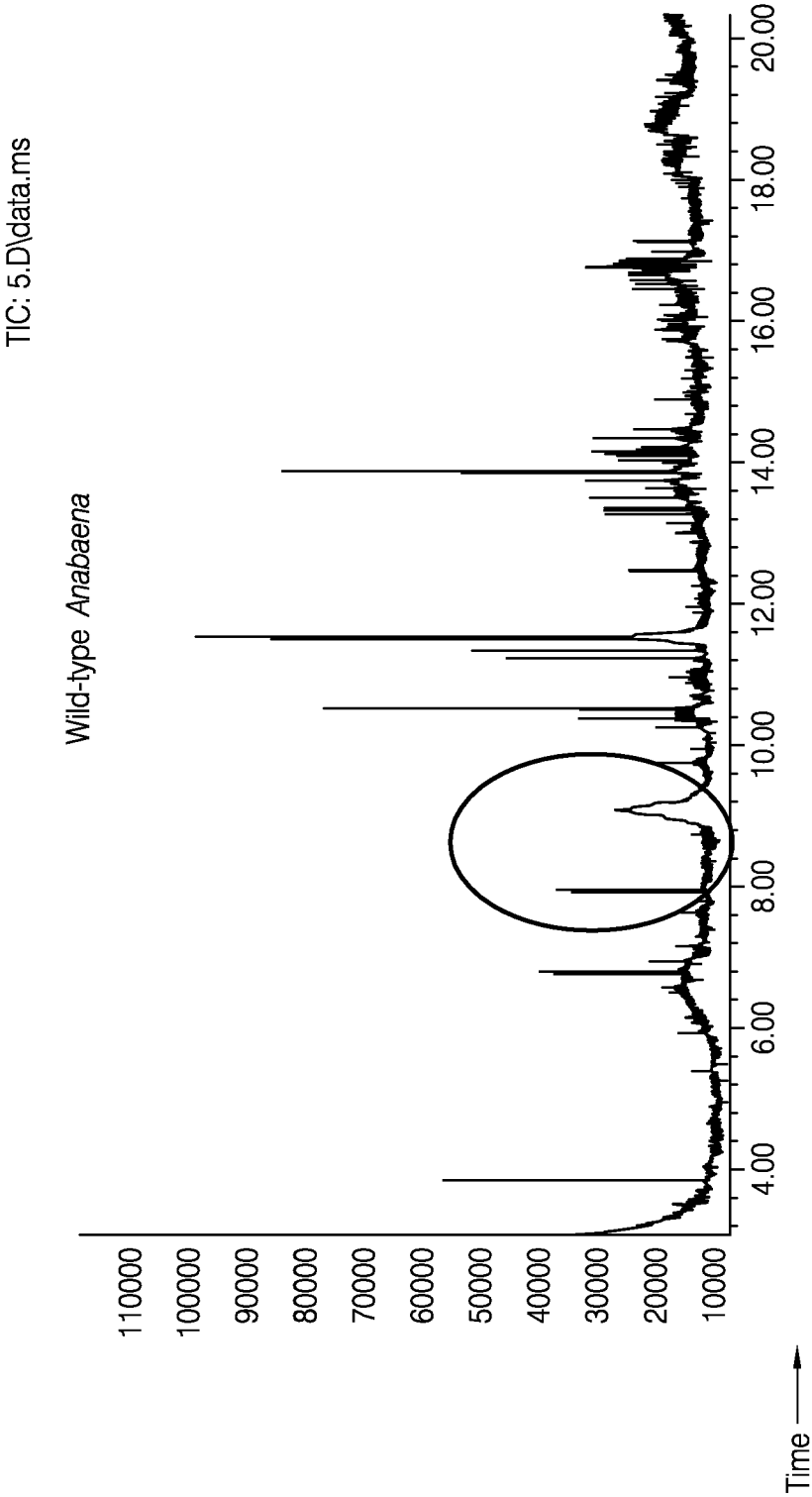


FIG. 6C

9/15

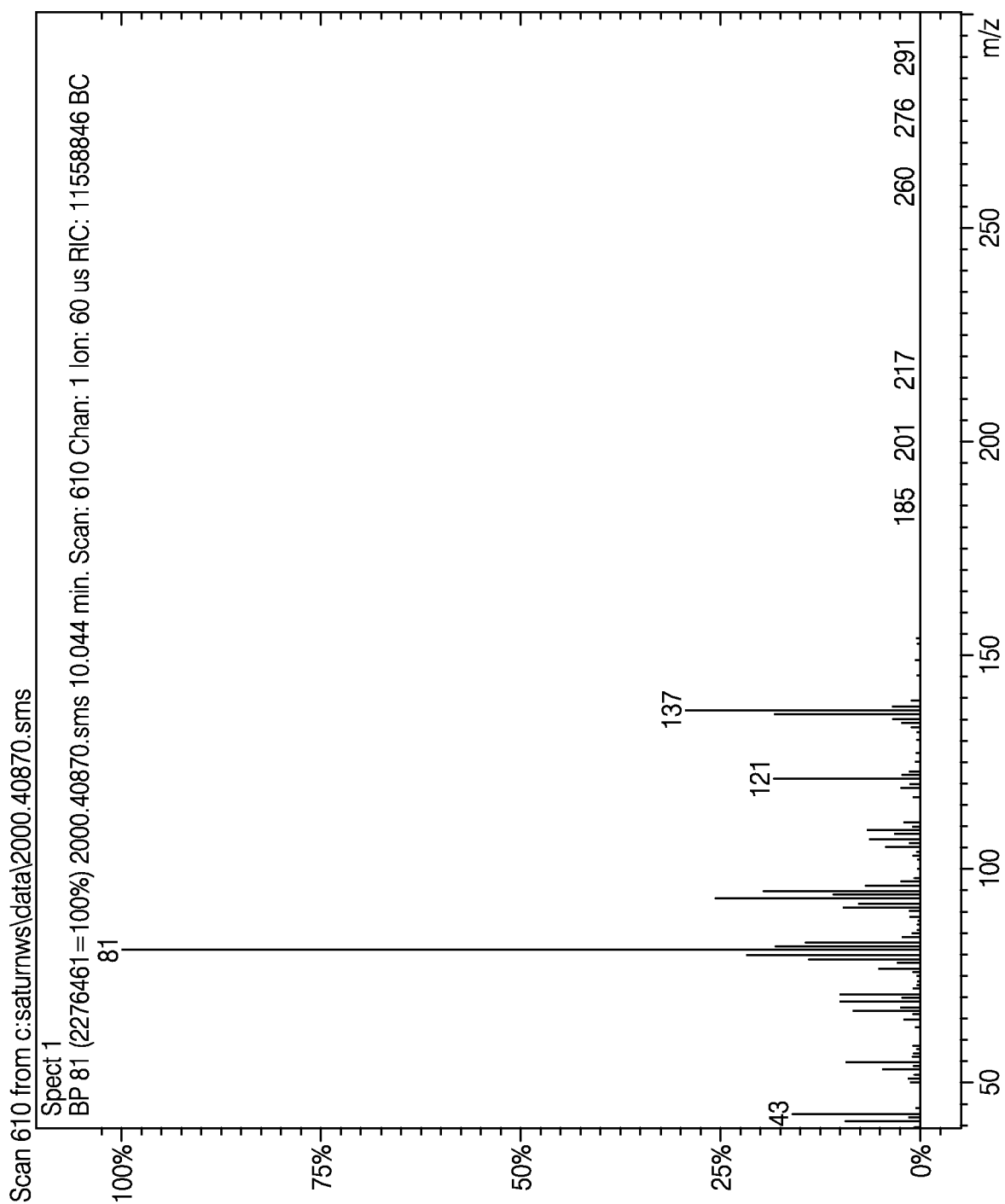


FIG. 7

10/15

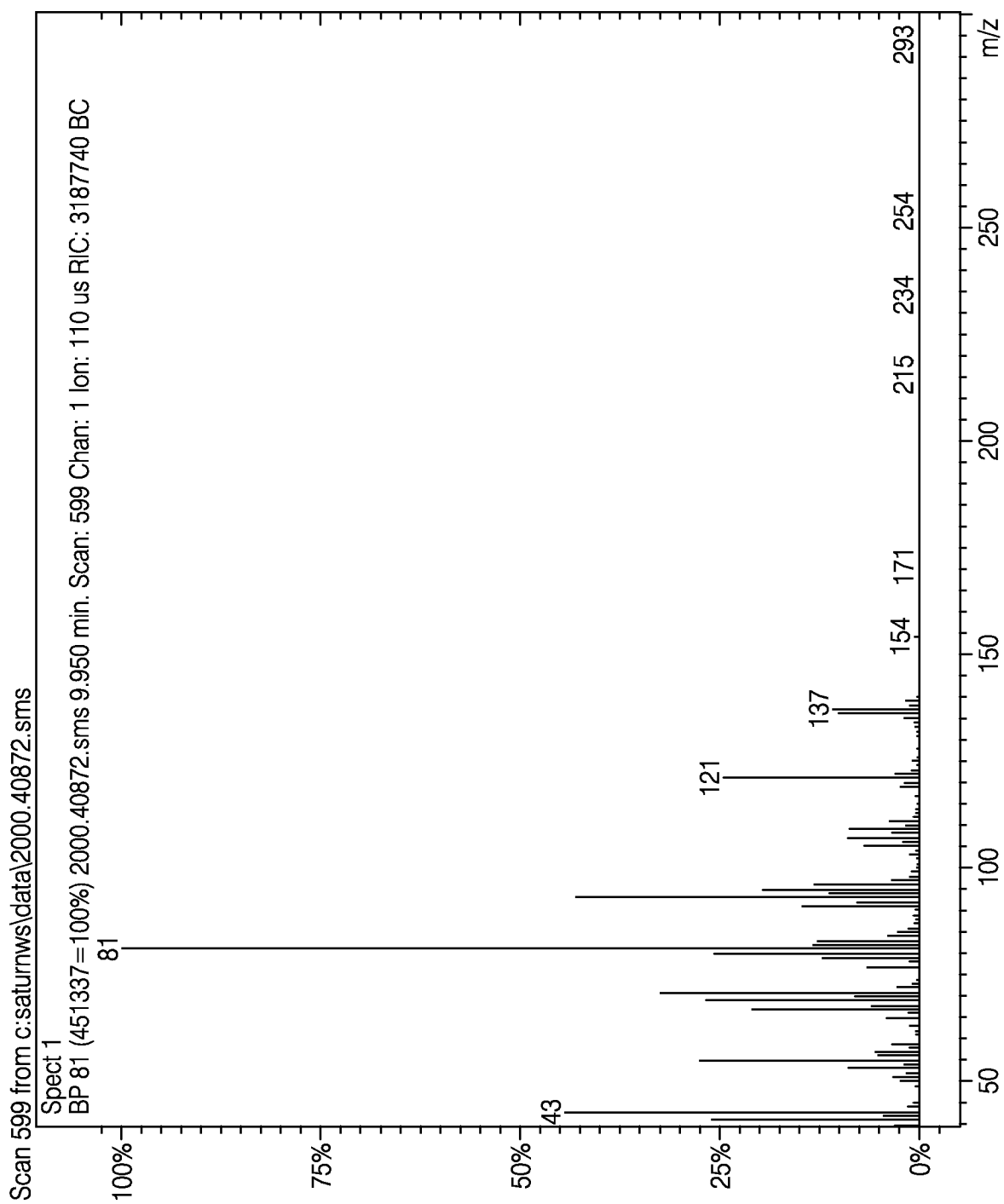


FIG. 8

11/15

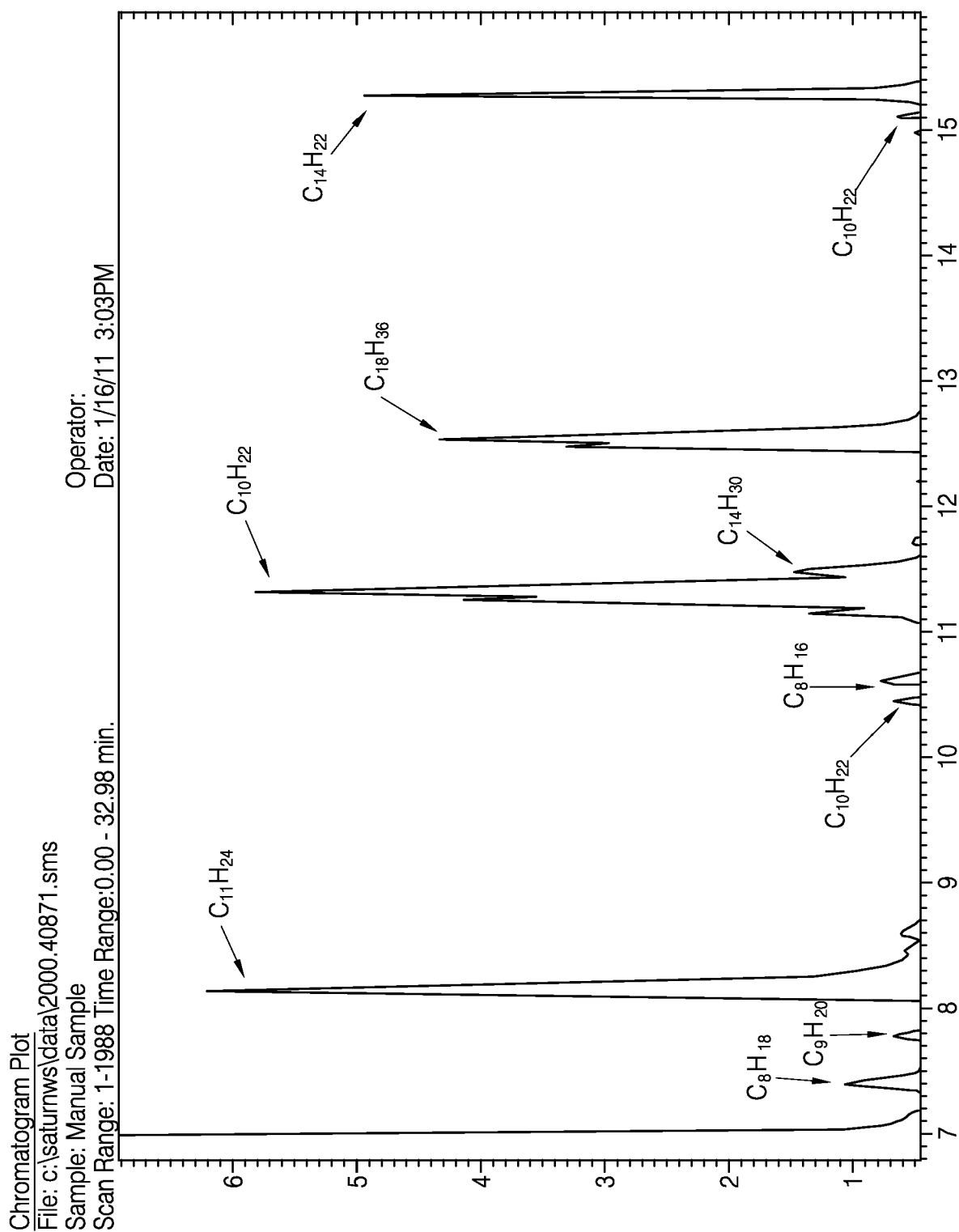
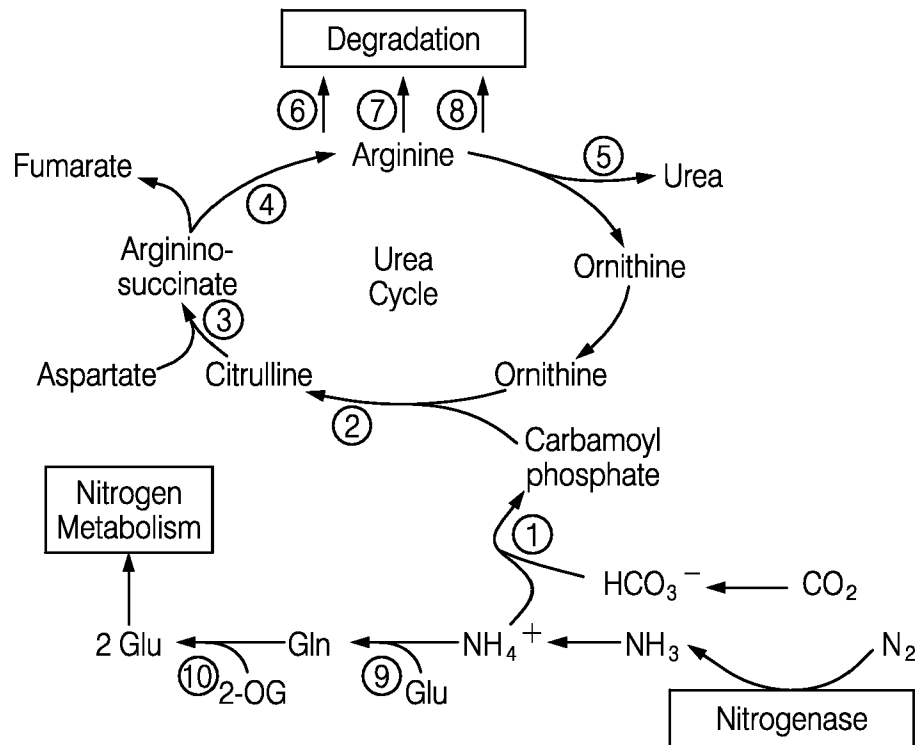


FIG. 9

12/15



- ① Carbamoyl phosphate synthetase-1 (CPS-1)
- ② Ornithine transcarbamoylase (OTC)
- ③ Argininosuccinate synthetase (ASS)
- ④ Argininosuccinase (AS)
- ⑤ Arginase
- ⑥ Arginine decarboxylase pathway
- ⑦ Arginine deiminase pathway
- ⑧ Arginine amidinotransferase pathway
- ⑨ Glutamine synthetase
- ⑩ Glutamate synthase

FIG. 10

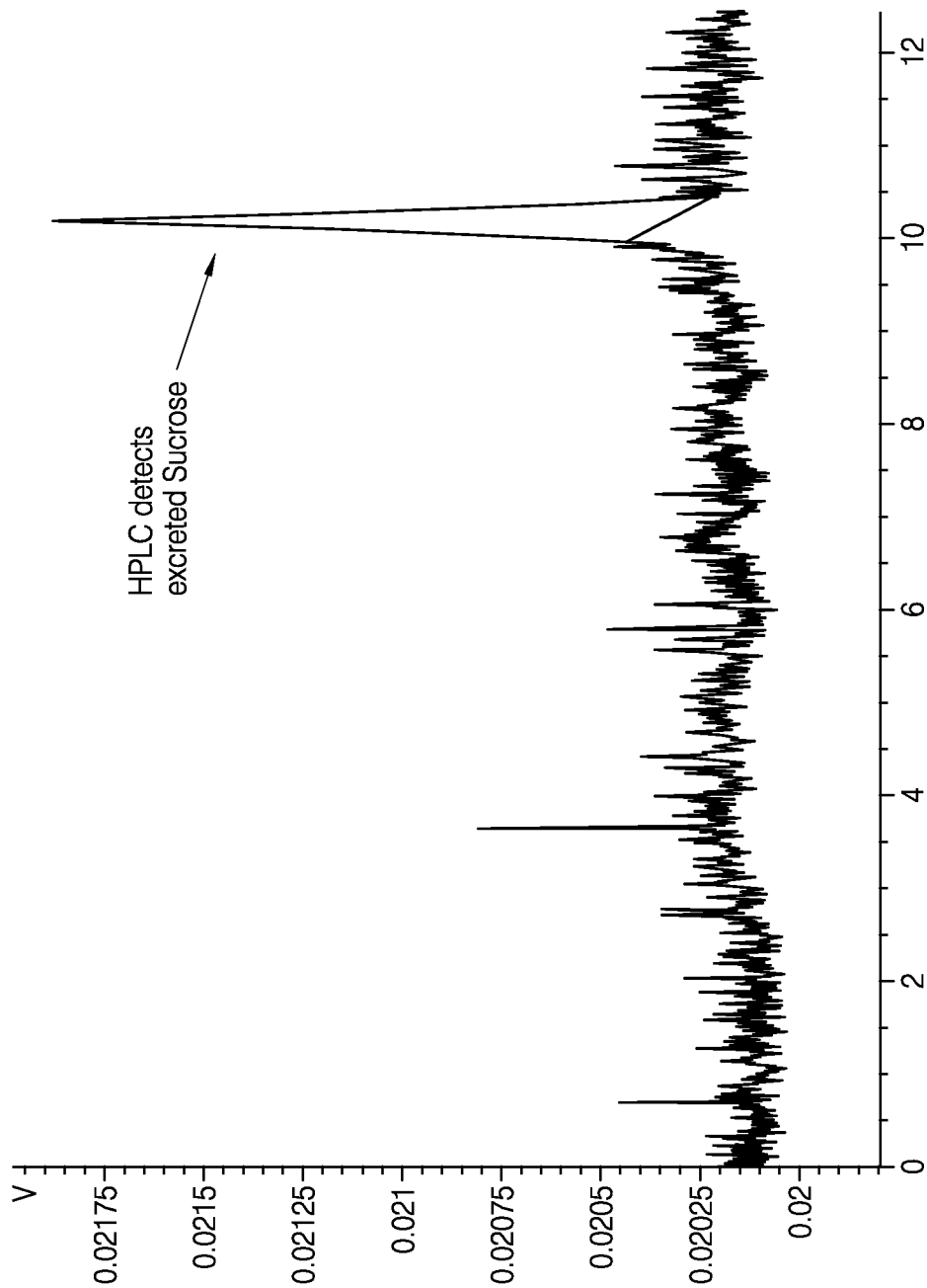


FIG. 11

14/15

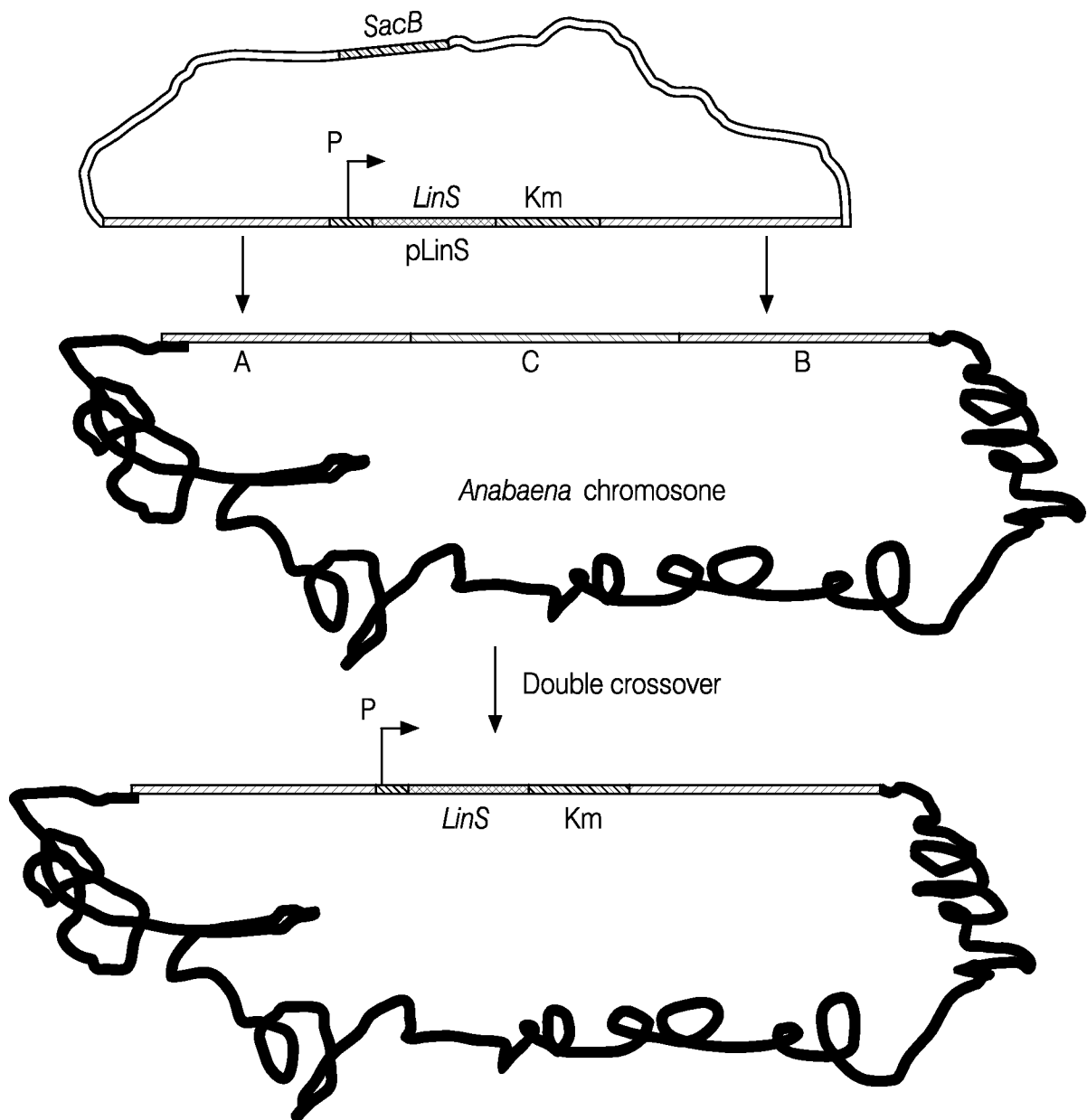


FIG. 12

15/15

Homologous genes from MEP pathway found in five cyanobacterial strains*

	<i>Arabidopsis thaliana</i>	<i>Anabaena</i> sp. PCC 7120	<i>Anabaena</i> <i>variabilis</i> ATCC 29413	<i>Thermosyne-</i> <i>chococcus</i> <i>elongatus</i> BP-1	<i>Synechocystis</i> sp. PCC 6803	<i>Nostoc</i> <i>punctiforme</i> ATCC 29133
DXS		alr0599	Ava_4532	tlI0623	sll1945	Npun_F5466
	AT3G21500 (DXS1)	1E-137	1E-138	1E-143	1E-139	1E-135
	AT4G15560 (DXS2)	1E-148	1E-149	1E-155	1E-149	1E-144
	AT5G11380 (DXS3)	1E-110	1E-110	1E-109	1E-103	1E-105
DXR	AT5G62790	alr4351	Ava_1300	tlr1040	sll0019	Npun_R5970
		1E-151	1E-151	1E-147	1E-145	1E-151
MCT	AT2G02500	all5167	Ava_2414	tlr0605	slr0951	Npun_F5020
		2E-28	8E-28	5E-32	8E-28	3E-26
CMK	AT2G26930	alr3230	Ava_4887	tlI0500	sll0711	Npun_R4911
		1E-22	2E-22	8E-22	5E-20	2E-21
MDS	AT1G63970	alr3883	Ava_1811	tlr2035	slr1542	Npun_F5826
		2E-38	2E-38	2E-38	6E-38	4E-38
HDS	AT5G60600	all2501	Ava_0433	tlr0996	slr2136	Npun_F5054
		2E-73	4E-73	9E-70	1E-72	1E-72
HDR	AT4G34350	all0985	Ava_2949	tlr1041	slr0348	Npun_R3286
		1E-148	1E-148	1E-145	1E-144	1E-142
IDI						
	AT5G16440 (IDI1)	None	None	None	None	None
	AT3G02780 (IDI2)	None	None	None	None	None
GPPS		alr0096	Ava_1469	tlr1757	slr0611	Npun_R1834
	AT1G78510 (GPPS1)	1E-95	4E-97	2E-98	4E-99	1E-95
	AT2G34630 (GPPS2)	2E-61	2E-62	2E-58	2E-61	2E-62
FPPS		alr0096	Ava_1469	tlI0020	slr0739	Npun_R1834
	AT5G47770 (FPPS1)	6E-04	2E-03	5E-06	5E-04	3E-04
	AT4G17190 (FPPS2)	4E-04	5E-05	5E-05	6E-03	1E-04
GGPPS		alr0213	Ava_2704	tlI0020	slr0739	Npun_F3770
	AT4G36810 (GGPPS1)	6E-89	2E-88	1E-99	7E-88	1E-88
	AT2G23800 (GGPPS2)	4E-76	3E-77	2E-78	2E-75	2E-80
	AT3G14550 (GGPPS3)	4E-79	6E-79	1E-84	3E-84	3E-81
	AT2G18640 (GGPPS4)	8E-76	1E-76	1E-79	7E-77	1E-79
	AT1G49530 (GGPPS6)	6E-67	1E-66	3E-65	7E-77	2E-68
	AT3G14530	2E-79	3E-79	9E-85	1E-85	5E-82
	AT3G32040	1E-77	8E-78	4E-81	4E-81	3E-79
SQS		alr1805	Ava_4808	tlI1096	sll0513	Npun_R2917
	AT4G34640 (SQS1)	3E-08	2E-07	2E-16	2E-09	2E-06
	AT4G34650 (SQS2)	4E-05	8E-05	1E-13	1E-07	1E-04
LinS	AT1G61680 (TPS14)	None	None	None	None	None

* *Arabidopsis* genes were used for Blast search, single gene found in each genome and its E-value included

FIG. 13