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(71) Applicant: **MATRIX GENETICS, LLC** [US/US]; 1600 Fairview Avenue E, Suite 300, Seattle, Washington 98102 (US).

(72) Inventors: **ROBERTS, James**; 1600 Fairview Avenue E, Suite 300, Seattle, Washington 98102 (US). **DOUGHTY, David M.**; 1600 Fairview Avenue E, Suite 300, Seattle, Washington 98102 (US).

(74) Agents: **WINGER, C. Rachal** et al.; Lee & Hayes, PLLC, 601 W. Riverside Avenue, Suite 1400, Spokane, Washington 99201 (US).

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(54) **Title**: MICROORGANISMS WITH BROADENED LIGHT ABSORPTION CAPABILITY AND INCREASED PHOTOSYNTHETIC ACTIVITY

(57) **Abstract**: Photosynthetic microorganisms with broadened light absorption capability and increased photosynthetic activity are described. Broadened light absorption is achieved by modifying the microorganism to utilize non-native bilins. Increased photosynthetic activity results from the broadened light absorption and can also result from a decrease in selfshading. The microorganisms include Cyanobacteria, including modified Cyanobacteria.

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MICROORGANISMS WITH BROADENED LIGHT ABSORPTION CAPABILITY AND INCREASED PHOTOSYNTHETIC ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This claims priority to U.S. Provisional Patent Application No. 62/191,171 filed on July 10, 2015, which is incorporated herein by reference in its entirety as if fully set forth herein.

FIELD OF THE DISCLOSURE

[0002] The disclosure provides microorganisms with broadened light absorption capability and increased photosynthetic activity. Broadened light absorption is achieved by modifying the microorganism to utilize non-native bilins. Increased photosynthetic activity results from the broadened light absorption and can also result from a decrease in self-shading. The microorganisms include Cyanobacteria, including genetically-modified Cyanobacteria.

BACKGROUND OF THE DISCLOSURE

[0003] Photosynthesis is a process by which solar energy is converted into chemical bond energy. The process of photosynthesis ultimately results in biomass accumulation. Biomass can be used to produce energy, fuel, chemicals, and food. As examples, bioethanol can be produced through alcohol fermentation of saccharified carbohydrate, and biodiesel oil and biojetfuel can be produced from neutral lipids such as waxesters and triglycerides. Further, photosynthesis processes environmental carbon dioxide.

[0004] Photosynthetic crops such as soy beans, corn, and palms have been used as raw materials to produce biofuel and other products. Use of edible crops for such purposes, however, can contribute to food shortages. Non-edible crops such as jatropha and camelina have also been used, but these crops have low yields per unit area.

[0005] Photosynthetic microorganisms similarly can be cultivated to produce energy, fuel, chemicals, and food, as well as to process environmental carbon dioxide. In fact, many of these photosynthetic microorganisms are capable of producing larger amount of oils, fats and carbohydrates than plants.

[0006] Phycobilisomes are protein and bilin complexes used by photosynthetic

microorganisms to capture light for photosynthesis. Optimal amounts of photosynthesis occur when every photon is used for photochemistry. However, natural photosynthetic microorganisms, such as cyanobacteria, utilize limited types of bilins, and often produce and utilize only one type of bilin. Because of these limited bilin types used by particular strains, each strain is a light harvesting specialist; that is, they preferentially use only a limited portion of the visible light spectrum for photosynthesis. *Synechococcus elongatus* 7942 (*Syn* 7942), for example, produces the bilin phycocyanobilin (PCB) and thus predominantly uses red/orange light for photosynthesis.

[0007] Photosynthesis also can be limited by self-shading. Self-shading occurs when photosynthetic microorganisms within a culture create obstacles to the passing of light to other photosynthetic microorganisms within the culture. Growth of the culture is inefficient or restrained when light no longer sufficiently passes through the thickness of the culture to reach all photosynthetic microorganisms within the culture in optimized amounts. Such self-shading can limit biomass yields.

SUMMARY OF THE DISCLOSURE

[0008] The current disclosure provides modified photosynthetic microorganisms with broadened light absorption capability and increased photosynthetic activity. Broadened light absorption capability is achieved by modifying photosynthetic microorganisms to utilize additional bilins so that they may more efficiently utilize additional portions of the light spectrum for photosynthesis. Increased photosynthetic activity is achieved by allowing use of additional portions of the light spectrum. Increased photosynthetic activity can also occur due to a decrease in self-shading caused by over expression of native bilins. These increases in photosynthetic activity have the potential to increase total carbon fixation, production of carbon containing compounds, and growth (biomass accumulation), among other uses. The current disclosure additionally provides bilin-binding proteins (i.e., phycobiloproteins) with red and purple pigments.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIG. 1 provides a schematic of selected pathways for phycocyanobilin (PCB), phycoerythrobilin (PEB), and phycourobilin (PUB) biosynthesis.

[0010] FIG. 2 shows cultures *Syn 7942* strain MX2037 before (left) and after (right) the

addition of IPTG. As can be seen in color reproductions, the before culture is a light emerald green while the after culture is a dark brown/black.

[0011] FIG. 3 shows absorption spectra of modified *Syn* 7942 strain MX2037 following induction by IPTG (dashed line) and a MX2037 culture uninduced (solid line). The strain utilizes PCB as its native bilin and was modified to utilize PEB as a non-native bilin.

[0012] FIG. 4 shows SDS-PAGE analysis of phycobilisome proteins. As can be seen in color reproductions, PCB is a blue pigment and PEB is a pink pigment. Following induction of PEB the α-subunit turns pink suggesting PCB has been replaced by PEB and the β-subunit turns purple suggesting it has bound a mixture of both PEB and PCB. The migration distances for the α- and β-subunits are labeled with arrows.

[0013] FIGs. 5A-5G show analysis of phycobilisome components of *Syn* 7942 in wild-type and modified strains utilizing PEB and PUB. Absorption spectra of allophycocyanin (dashed lines) and phycocyanin (solid lines) in (5A) wild type, (5B) PEB utilizing strain (MX2037), and (5C) PUB utilizing strain (2064). Photo showing the color of allophycocyanin and phycocyanin in the different strains (5D). As can be seen in color reproductions of the data, wild type pigments are blue, phycocyanin in the PEB utilizing strain is purple, phycocyanin in the PUB utilizing strain is dark blue, and allophycocyanin remains blue in all strains independent of PEB and PUB utilization. Visual (5E), zinc acetate stained (5F), and Imperial blue (5G) analysis of proteins after SDS-PAGE. As can be seen in color reproductions, zinc acetate causes PCB to fluoresce red, PEB to fluoresce yellow, and PUB to fluoresce green.

[0014] FIGs. 6A-6E show the separation of the alpha and beta subunit of phycocyanin by LC/MS (6A). The absorption spectra of wild type shows that PCB is bound to the alpha (6B) and beta subunits (6C) as indicated by peak absorbance near 625 nm. The absoption spectra of induced MX2037 shows that PEB is bound to the alpha subunit (6D) and that both PCB and PEB are bound to the beta subunit (6E).

[0015] FIGs. 7A-7D show identification of PEB by LC/MS. The relative abundance of the 587 molecular ion of PEB (7A) and isomers of PEB are indicated by arrows. The absorption spectra of PEB showing the expected 626 absorbance maximum (7B). Chromatograms of the PEB isomers (7C and 7D).

[0016] FIGs. 8A and 8B show green light titrations in the uninduced (dashed line) and

induced (solid line) PEB utilizing strain (8A) and the data calculated as a percent increase (8B).

[0017] FIGs. 9A and 9B show absorption spectra of a culture induced (dashed line) to utilize PEB and an uninduced (solid line) culture (9A). The solid arrow indicates the expected absorption maximum of PEB and the outlined arrow indicates the expected absorption value of PCB. Oxygen evolution in response to red light (9B) in the PEB utilizing strain, the wild type, and uninduced cultures. Oxygen evolution in response to yellow/amber light (9C) in the PEB utilizing strain, the wild type, and uninduced cultures.

[0018] FIG. 10 shows absorption spectra of the water soluble fraction of whole cell lysate from wild type (solid line), induced for PEB utilization (dashed line), and induced PebAB + RpcG (dotted line) cultures. The expected absorption maximum for phycourobilin is indicated by the arrow.

[0019] FIG. 11 shows absorption spectra of water soluble cell lysate from the wild-type (dotted line) PebAB utilizing strain (solid line), and the Δ*cpcE* + *pebAB* +*rpcG* (dashed line). The arrow indicates the region of light expected to be absorbed by PUB.

[0020] FIGs. 12A-12C show SDS-PAGE analysis of bilins using visible light (12A), Zn acetate stain (12B), and protein stain (12C). As can be seen in visual reproductions of the data, zinc acetate staining causes red fluorescence of PCB, yellow fluorescence of PEB, and green fluorescence of PUB.

[0021] FIGs. 13A-13C show the separation of the alpha and beta subunit of phycocyanin by LC/MS in the MX2064 mutant (13A). The absorption spectra of the alpha subunit (13B) shows two absorbance peaks 493 nm is the expected absorbance of PUB and 550 is the expected absorbance of PEB. The beta subunit (13C) shows maximal absorbance at 603 nm which does not correspond to the known absorbance spectras of PCB, PEB, or PUB.

[0022] FIG. 14 shows oxygen evolution rates in the induced and uninduced PUB utilizing strain (MX2064) in response to blue light. All data points are the mean of three replicates and error bars represent one standard deviation.

[0023] FIGs. 15A and 15B show LC/MS analysis of the wild type (15A) and PUB utilizing strain (15B). Brackets label the regions in which new peaks appear.

[0024] FIGs. 16A and 16B show spectra of the α-subunit of the MX2479 mutant in which pebA, pebB, rpcG, and cpcAB are expressed from inducible promoters (PEB strain; 16A;

samples 5 and 6; 16B). The cpcA gene encodes a mutation T130C.

[0025] FIGs. 17A-17C show wild type (17A), PEB (MX2037; 17B) and PUB+PEB (MX2064; 17C) functioning in exciton transfer to PSII using 77K fluorescence.

[0026] FIGs. 18A-18C show increased growth rate (18A; 18B) and dry weight yield (18C) of the MX2479 (Y130C) mutant.

[0027] FIGs. 19A and 19B show increased oxygen evolution of the MX2479 (Y130C) and MX2507 (A26K) mutants at white light titration (19A) and at the 100 μE illumination point (19B).

[0028] FIGs. 20A-20C show a comparison of oxygen evolution in response to different wavelengths of light. The *cpeS* (MX2506; 20B) and Y130C (MX2479; 20C) mutants show increase oxygen evolution in response to 520 nm and 505 nm light compared to the wild type (20A).

[0029] FIG. 21 shows an amino acid alignment of bilin binding protein fragments that bind to PCB (CpcA, CpcB, ApcA, ApcB); PEB (CpeA); and PUB (RpcA, and MpeA). α-subunits of phycocyanin (e.g., CpcA (2 homologs); RpcA (2 homologs), etc.) and allophycocyanin (ApcA (6 homologs) are provided above β-subunits of phycocyanin and allophycocyanin. The arrow indicates the position of the universally conserved cysteine (position 82/84) that binds bilin pigments.

[0030] FIG. 22 provides exemplary protein and nucleotide sequences supporting the disclosure.

DETAILED DESCRIPTION

[0031] Photosynthesis is a process by which solar energy is converted into chemical bond energy. The overall reaction of photosynthesis is the light-driven conversion of carbon dioxide and water to glucose and oxygen:

$6CO_2+6H_2O \rightarrow C_6H_{12}O_6+6O_2$

Photosynthesis is observed in plants as well as in bacteria, and blue-green algae.

[0032] The process of photosynthesis ultimately results in biomass accumulation. Biomass can be used to produce energy, fuel, chemicals, and food. As examples, bioethanol can be produced through alcohol fermentation of saccharified carbohydrate, and biodiesel oil and biojetfuel can be produced from neutral lipids such as waxesters and triglycerides. Further, photosynthesis processes environmental carbon dioxide.

[0033] Cyanobacteria have developed a number of pigmented proteins to collect light energy optimally for photosynthesis. Most utilize finely tuned antennae known as phycobilisomes, which are supramolecular structures composed of both chromophorylated and non-chromophorylated proteins. The chromophorylated components, i.e., the phycobiliproteins, carry covalently bound, linear tetrapyrroles (bilins) that are responsible for the light-harvesting properties of these proteins. Cyanobacteria can utilize at least four different types of bilins: phycocyanobilin (PCB); phycoerythrobilin (PEB); and their respective Δ5-to-Δ2 double-bond isomers, phycoviolobilin (PVB) and phycourobilin (PUB). Each bilin is able to absorb a different portion of the visible light spectrum. For example, PCB absorbs light in the orange/red spectrum (a wavelength of 550 to 650 nm); PEBs absorb light in the green spectrum (a wavelength of 525 to 590 nm); PUB absorbs light in the blue spectrum (a wavelength of 490 to 525 nm); and PVBs absorb light in the yellow light spectrum (a wavelength of 550 to 590 nm).

[0034] Optimal amounts of photosynthesis occur when every photon is used for photochemistry. However, natural photosynthetic microorganisms such as cyanobacteria are light harvesting specialists because they typically do not utilize every bilin type. For example, the cyanobacterial strain *Synechococcus elongatus* 7942 (*Syn* 7942) naturally utilizes only the PCB bilin and accordingly predominantly uses red/orange light for photoynthesis. In contrast, *Syn* 8102 utilizes predominantly green light absorbing PEB and predominantly blue light absorbing PUB (Blot et al., (2009) *J. Biol. Chem.*, 284(14):9290-8. doi: 10.1074/jbc.M809784200).

[0035] Photosynthesis also can be limited by self-shading. Self-shading occurs when photosynthetic microorganisms within a culture create obstacles to the passing of light to other photosynthetic microorganisms within the culture. Growth of the culture is inefficient or restrained when light no longer sufficiently passes through the thickness of the culture to reach all photosynthetic microorganisms within the culture in optimized amounts. Such self-shading can limit biomass yields.

[0036] The current disclosure examined whether photosynthetic microorganisms could be modified to utilize bilin types in addition to those that they predominantly use. To assess this question, photosynthetic microorganisms were modified to express non-native bilins

in addition to the particular organism's native bilin type and whether expression of an additional non-native bilin would broaden the range of light used for photosynthesis and increase photosynthetic activity (e.g., expand the active photosynthetic spectrum) was assessed. "Non-native" means a compound (e.g., bilin, protein, nucleotide sequence) that is not naturally produced by an unmodified photosynthetic microorganism, or, if produced, is produced at a significantly different level or for a significantly different purpose than its introduced counterpart. Before describing the methods and results in detail, background information regarding bilin synthesis is provided.

[0037] A schematic of selected bilin synthesis production pathways is provided in FIG. 1. A cell produces heme which is subject to a heme oxygenase (e.g., HO1) to form a biliverdin. The biliverdin is further subject to a bilin reductase (and may be further subject to additional enzymes of the cell such as additional reductases), to form the required bilins. Bilins are then joined to the required phycobiliprotein by a lyase. Note, however, that certain strains utilize proteins that can serve more than one function. For example, RpcG (SEQ ID NO: 1) is a fusion protein with enzymatic and lyase functions that can be found in strains utilizing PUB. Moreover, certain strains utilize phycobiliproteins with an auto-lyase activity. That is, a lyase independent of the phycobiloprotein is not needed. Accordingly, and as will be understood by one of ordinary skill in the art, in practicing the teachings of the current disclosure, one must take into consideration the strains being used and their particular bilin synthesis and utilization protocols. Following the teachings of the current disclosure, some experimentation may be required to optimize expansion of the active photosynthetic spectrum in particular strains. The current teaching is more than sufficient, however, to allow one of ordinary skill to succeed in producing a modified photosynthetic microorganism with an expanded active photosynthetic spectrum.

[0038] An exemplary biliverdin reductase includes 3Z-phycocyanobilin:ferredoxin oxidoreductase (PcyA) which converts biliverdin to PCB. More particularly, PcyA performs a two-step reaction: the reduction of the vinyl pyrrole A ring of biliverdin IXα and the reduction of the 18-vinyl group to yield PCB. Additional examples of biliverdin reductases include the 3Z-phycoerythrobilin:ferredoxin oxidoreductases, PebA (SEQ ID NO: 2), PebB (SEQ ID NO: 3) and PebS. Often PebA and PebB are found in the same pathway. The PebA-PebB pathway is found in many cyanobacteria, which uses PebA to reduce

biliverdin IXα to 15,16 dihydrobiliverdin (DHBV), and then uses PebB to reduce 15,16 DHBV further to PEB. Alternatively, PebS from the myovirus P-SSM4 can perform both reactions in a manner similar to the two-step reduction of biliverdin to PCB by PcyA.

[0039] Generally, bilins are attached to phycobiloproteins by lyases. Exemplary lyases include CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, and PecF. CpcE/CpcF, is a heterodimer responsible for attachment of PCB to CpcA. The related lyase, PecE/PecF, catalyzes the formation of PVB from PCB and attaches this bilin to PecA. Other characterized lyases include the CpcS or the CpcS/CpcU heterodimer lyases, both of which can attach PCB to conserved Cys residues at the 82/84 positions of CpcB, ApcA, ApcB, ApcD, and ApcF; and the CpcT lyase, which attaches PCB to Cys153 residue of CpcB. RpcG, which appears to be a fusion of PecE- and PecF-type domains, has been reported to attach both PVB and PUB to RpcA, its cognate apoprotein. Lyases which are homologous to the CpcS/CpcU/CpcT family and are likely to be responsible for the attachment of PEB in phycoerythrins, have been identified in the genomes of numerous species. These enzymes are largely uncharacterized but are likely to function similarly. Phycoerythrins have more bilins than phycocyanins, and thus they are likely to require additional lyases for their post-translational maturation. Also note that PVB and PUB do not occur as free bilins in cyanobacteria cells; instead, these two bilins are formed by isomerizing lyases that convert PCB and PEB to PVB and PUB, respectively, and attach them to cysteines of the appropriate phycobiloproteins.

[0040] As noted, bilin lyases in addition to CpcE and CpcF include PecE and PecF, which catalyze the addition of PCB to the phycoerythrocyanin apo-α subunit and the isomerization of the bound bilin to phycobiliviolin (Jung, et al.,. (1995) *J. Biol. Chem.*, 270, 12877-12884; Zhao, et al., (2000) *FEBS Lett.*, 469, 9-13). CpeY plus CpeZ have been reported to catalyze the addition of phycoerythrobilin to one of the bilin attachment sites on the α subunit of C-phycoerythrin (Kahn, et al., (1997) *J. Bacteriol.*,. 179, 998-1006). The lyase may provide any required isomerase activity, or such activity may be provided by an independent isomerase, which may be endogenous or recombinant.

[0041] Because most cyanobacteria only naturally utilize one type of native bilin and one type of native bilin-binding protein (phycobiliprotein), the possibility that cyanobacteria could be modified to utilize additional bilin types was explored. More particularly, the

possibility that the heterologous expression of non-native proteins leading to the production of non-native bilins would lead to binding with a heterologous native phycobiliprotein and active light harvesting for photosynthesis, thus expanding the ability of a cyanobacteria to absorb light for photosynthesis to a broader region of the spectrum was explored. Even more particularly, the ability of two non-native bilins to enhance photosynthesis in *Syn* 7942 was explored.

[0042] In *Syn* 7942 only the predominantly orange/red light absorbing bilin, PCB, is produced, and therefore maximal rates of photosynthesis are predominantly observed in orange/red light. Other cyanobacteria produce bilins that predominantly absorb green (PEB) and/or blue (PUB) light. For example, the *pebA* and *pebB* genes with stop codons (e.g., SEQ ID NO: 4 and SEQ ID NO: 5, respectively), encoding the pathway for PEB biosynthesis, were cloned into *Syn* 7942 and placed under the control of an IPTG inducible promoter (for the entire inserted sequence, see, SEQ ID NO: 6 which includes or encodes *lacI*, *pebA*, *pebB*, and the streptomycin resistance cassette; SEQ ID NO: 6 was inserted into neutral site 1; see also SEQ ID NO: 9). When grown in the presence of IPTG, cultures turned dark compared to the uninduced control (FIG. 2). The production of PEB was confirmed by the presence of an absorption peak at 550 nm (FIG. 3) and the binding of PEB to the protein components of the phycobilisome were confirmed visually by SDS-PAGE analysis (FIG. 4). Because, PCB is blue under visual light and PEB is pink, visual inspection of the protein gel provided information regarding the localization of PEB to the various subunits of the phycocbilisome. In the uninduced control both the α- and β-subunits of phycocyanin were blue because of bound PCB. Following induction of PEB the α-subunit turned pink suggesting PCB had been replaced by PEB and the β-subunit turned purple suggesting it had bound a mixture of both PEB and PCB. Note that the ability to express alternatively pigmented phycocyanin (red and purple) is an unexpected utility of the disclosure, because phycocyanin is already highly valued as a natural blue pigment, and red and purple versions have similar value. Further information regarding the binding of PEB to phycobilisome proteins was gained by the separation of the two types of bilin binding proteins, allophycocyanin and phycocyanin. Allophycocyanin precipitates at 40% ammonium sulfate and has an absorption maximum at 650 nm. Following the induction of PEB, allophycocyanin did not show an absorbance peak at 550

suggesting it did not bind PEB. Instead the PEB absorbance maximum was specifically observed in the phycocyanin fraction (FIG. 5). The binding pattern of bilins was explored using LC/MS to separate the alpha and beta subunits of phycocyanin (Kumar et al. (2014) Ind J Plant Physiol. 19, 184-188). PEB was found to be the only pigment on the alpha subunit and the beta subunit contained both PEB and PCB in approximately equal abundance (FIGs. 6A-6E). Finally to ensure that the observed bilin was PEB, its identity was confirmed using LC/MS. PEB can be extracted from phycocyanin as 2 isomers both with parent ions of 587 and absorption maximums at 626 nm (FIGs. 7A-7D and Fu, *et al.*, (1979), *J. Biochem.*,. 179:1-6).

[0043] To explore the effects of PEB expression on photosynthesis, a green light titration was performed. When green light was supplied for photosynthesis, the PEB producing strain always produced more oxygen than the uninduced control (FIGs. 8A, 8B). The PEB expressing strain was very sensitive to low levels of green light (100 µE) and showed up to an 80% improvement over the uninduced control.

[0044] Because PEB expression corresponded to a decrease in PCB (FIG. 9A) and over expression of PCB has been shown to cause self-shading in red light, the possibility that PEB expression would reduce red light self-shading was explored. When cells were suspended to an OD_{750} of 2 and provided 500 µE of red light (630 nm), cultures that produced PEB showed a 16% improvement in oxygen evolution compared to the wild-type or uninduced control (FIG. 9B).

[0045] As a control, the experiment was repeated at a wavelength of light where PEB expression would not be expected to affect the absorption of light. When 590 µE of yellow light (590 nm) was provided the PEB expressing strain showed the same rates of oxygen evolution as the wild type and uninduced controls (FIG. 9C).

[0046] The heterologous expression of PUB in *E. coli* has been reported (Alvey, et al., (2011a), *Biochemistry*, . 50(22):4890-902. doi: 10.1021/bi200307s). As disclosed herein, a strain of *Syn* 7942 was modified to express all of the necessary components for PUB biosynthesis and attachment to phycobilisomes. SEQ ID NO: 7 represents a nucleotide sequence encoding RpcG with a stop codon. SEQ ID NO: 8 was inserted into neutral site 4 of *Syn* 7942 and includes or encodes *lacI*, *rpcG*, and the gentamycin resistance cassette. SEQ ID NO: 9 was inserted into neutral site 3 and includes or encodes paraup1,

pebA, *pebB*, and the hygromycine resistance cassette. Surprisingly, no PUB was observed in *Syn* 7942 (FIG. 10) following induction of expression.

[0047] Because bilins generally are attached to phycobiloproteins (e.g., phycocyanin) by the activity of lyase enzymes and the final enzymatic step in PUB biosynthesis is carried out by the RpcG fusion protein (that has both lyase and biosynthetic roles), it was reasoned that competition between the native lyase, CpcE, and the introduced lyase RpcG could be prohibiting the expression of PUB. Construction of a strain of *Syn* 7942 in which cpcE was deleted (SEQ ID NO: 10 represents the deleted sequence) and replaced with SEQ ID NO: 11 and the PUB biosynthetic pathway was expressed (SEQ ID NOs: 8 and 9) resulted in a strain of *Syn* 7942 in which PUB was expressed (MX2064) (FIG. 11). The binding of PUB specifically to the phycocyanin α-subunit was confirmed by SDS-PAGE analysis of proteins and a zinc acetate strain in which the phycocyanin fluoresced red when bound by PCB, yellow when bound by PEB, and green when bound by PUB (FIG. 12). LC/MS also allowed for the separation of the alpha and beta subunits of phycocyanin. The α-subunit of phycocyanin was found to bind a mixture of PEB and PUB while the β-subunit was found to bind an unknown bilin with a maximum absorption of 603 nm (FIGs.13A-13C). Following induction, this mutant displayed a 66% increase in photosynthesis in response to blue light (FIG. 14) and continued to conduct photosynthesis in response to red light.

[0048] An unexpected result was that LC/MS analysis of the PUB producing strain indicated that a greater diversity of bilins was being produced than just PCB, PEB, and PUB (FIG. 15). Without being bound by theory, these likely are bilin isomers derived from PCB, PEB and/or PUB. In this connection, previous research has described the ability of the apo-phycocyanin α-subunit, in the absence of the CpcE lyase, to convert PCB into mesobiliverdin and an identified bilin via an isomerization reaction. By analogous reactions PEB and PUB could be isomerized into new uncharacterized bilins. Because apo-phycocyanin is the likely cause of the new bilin isomers, a site directed mutagenesis approach was used to limit this reaction and enhance the PUB binding properties of the phycocyanobilin α-subunit. A86K (MX2507; SEQ ID NO: 61) and Y130C (MX2479; SEQ ID NO: 62) variant forms of cpcA expressed as the cpcAB operon from an IPTG inducible promoter in neutral site 2 expressed small amounts of PUB on the alpha-subunit of

phycocyanin when expressed along with PebA, PebB, and RpcG, as detected by LC/MS (FIGs. 16A, 16B; see SEQ ID NOs. 57 and 58).

[0049] A second approach to solving the bilin isomerization problem was also taken. Because Syn 8102 does not form mesobiliverdin and its genome contains a family of bilin lyases known to attach bilins to the beta subunit of phycocyanin, the possibility that the expression of cpeS lyases could prevent mesobiliverdin formation in the MX2064 (ΔcpcE, RpcG, PebAB) background was explored. Induction of strains containing cpeS showed decreased mesobiliverdin on the beta subunit of phycocyanin (see, e.g., SEQ ID NOs. 59, 60, 69 and 70). Data are consistent with cpeS attaching PCB to the beta subunit of phycocyanin and preventing the formation of mesobiliverdin.

[0050] The functional expression of PEB and PUB was established using 77K fluorescence (FIG. 17A-17C). When an excitation light of 520 nm was applied to an induced culture of MX2037 PSII fluorescence was observed indicating that excitons had been transferred from PEB to PSII. Similarly, when an excitation light of 470 nm was applied to induced cultures of MX2064 PSII fluorescence was again observed indicating that excitons had been transferred from PUB to PSII.

[0051] Because PEB and PUB were functionally expressed in *Syn* 7942, and the mutant strains of *Syn* 7942 (A86K and Y130C) could express PUB without producing bilin isomers, the effects of PEB and PUB on growth were explored. At 400 μE of white LED light and 4% CO_2 the Y130C strain out grew the WT strain by 16% as measured by dry weight (Figure 18). White light titrations indicated that PUB expressing strains of S7942 had increased photosynthesis, as measured by oxygen evolution, at low light conditions of 100 μE (Figure 19). Action spectra in which 100 μE of light from each wavelength of light indicate that strains expressing PEB and PUB had enhanced oxygen evolution at light wavelengths of 520 and 505 nm (Figure 20). These data indicate that PUB and PEB expression enhance oxygenic photosynthesis in *Syn* 7942.

[0052] Several of the described results were unexpected. First, it was expected that both non-native phycobilisome protein components as well as non-native bilin biosynthetic enzymes would be required for the functional production and utilization of PEB and PUB. Instead it was found that the native phycocyanin of *Syn* 7942 could function with non-native bilins. In addition, the native lyase activities found in *Syn* 7942 could attach non-

native PEB to native phycocyanin. Second, it has never been previously demonstrated that production of a non-native bilin could increase photosynthesis in response to specific wavelengths of visible light (e.g., expand the active photosynthetic spectrum). Third, production of non-native PEB decreased native PCB production and relieved self-shading in red light. Although decreases in self-shading have previously been attributed to decreased PCB, this is a novel approach in which PCB reduction was achieved by funneling bilin metabolism into an alternative non-native bilin. Fourth, previous work showed that heterologous production of PUB in *E. coli* was enabled by the expression of the biosynthetic enzymes (PebA, PebB, RpcG), the phycocyanin protein (CpcA), and the lyase activity of RpcG (Blot, *et al.,* (2009), *J. Biol. Chem.*, 284(14):9290-8. doi: 10.1074/jbc.M809784200). The expression of all of these genes in *Syn* 7942 was insufficient to enable PUB production. Without being bound by theory, this is likely because the native pathways for bilin biosynthesis and attachment to phycocyanin were in competition with the non-native pathway including PebA, PebB, and RpcG that had been expressed in *Syn* 7942. By down-regulating native lyase CpcE that attaches native PCB to the native α-subunit of phycocyanin and complementing the down-regulation of native CpcE by expressing non-native RpcG, the competition between these pathways was reduced and PUB could be produced. Fifth, unexpected and perhaps previously unknown bilin isomers appeared in the PUB producing strain. Optimization of PUB function may require site directed mutagenesis of the native phycocyanin in order to make phycocyanin a better binding partner for PUB. Alternatively, mutagenesis could favor the production of one bilin isomer over the others.

[0053] Because two functional non-native bilins were heterologously produced, a variety of approaches and strategies that will be useful for the heterologous production of non-native bilins in other cyanobacteria were learned and are disclosed herein. Application of the teachings of the disclosure to other photosynthetic microorganisms and cyanobacteria is guided by the specific phycobilisome proteins and the specific endogenous lyase genes, which can vary widely among photosynthetic microorganisms including cyanobacteria. The consensus amino acid sequence for the PCB attachment site in phycocyanin is (A/S)(K/A)C(I/L/A)RD (SEQ ID NO: 12). Lyases which recognize this class of attachment site will also add non-native PEB to phycocyanin, if non-native PEB is

present. Exemplary species of cyanobacteria which express phycocyanin containing this consensus attachment site include *Syn* 7002 and *Synechocystis* 6803 and are candidates for non-native PEB production and/or incorporation into phycobilisomes and thus expansion of the active photosynthetic spectrum.

[0054] Functional utilization of PUB can require that a non-native lyase enzyme replace one of the native lyases that conjugates native PCB to a specific site on native phycocyanin; in examples described herein, this was CpcE. This approach could be broadly applicable to other cyanobacteria species including *Syn* 7002 and *Synechocystis* 6803, which contain homologues of CpcE and to other cyanobacteria species which express functionally equivalent lyases that control the attachment of PCB to specific sites on phycocyanin. An alternative approach is to engineer phycocyanin to replace its consensus PCB attachment site with a consensus PUB attachment site: (A/S)(K/A)CSRD (SEQ ID NO: 13). This would increase attachment of non-native PUB to a now non-native phycocyanin by a non-native RpcG lyase. These results and teachings demonstrate that photosynthetic microorganisms can be modified to functionally utilize non-native bilins.

[0055] Photosynthetic Microorganisms. Photosynthetic microorganisms of the disclosure may be any type of organism capable of performing photosynthesis wherein the microorganism has been modified to utilize a non-native bilin to broaden its light absorption capability, expand its active photosynthetic spectrum and increase photosynthetic activity. “Broaden its light absorption capability” means that the microorganism absorbs a wavelength of the visible spectrum that it does not absorb in its non-modified state or that it shows a significant increase in absorption of a wavelength of the visible spectrum over its non-modified state. The broadening of light absorption capabilities can increase photosynthetic activity by expanding a microorganism’s active photosynthetic spectrum. That is, the newly-absorbed light leads to increased photosynthetic activity.

[0056] Generally, naturally-occurring photosynthetic microorganisms utilizing native PCB absorb light predominantly in the orange/red spectrum with (a peak absorption occurring at 620 nm); naturally-occurring photosynthetic microorganisms utilizing native PEB absorb light predominantly in the green spectrum with (a peak absorption occurring at 550 nm); naturally-occurring photosynthetic microorganisms utilizing native PUB absorb

light predominantly in the blue spectrum with (a peak absorption occurring at 495 nm); and transgenic photosynthetic microorganisms utilizing PVB absorb light predominantly in the yellow light spectrum with (a peak absorption occurring at 570 nm). As previously noted, the methods disclosed herein can also increase photosynthetic activity by reducing shelf-shading.

[0057] Exemplary photosynthetic microorganisms that are either naturally photosynthetic or can be engineered to be photosynthetic include bacteria (*e.g.*, Cyanobacteria); fungi; archaea; protists; eukaryotes, such as a green algae; and animals such as plankton, planarian, and amoeba. Examples of naturally occurring photosynthetic microorganisms include *Arthrospira (Spirulina) maxima, Arthrospira (Spirulina) platensis, Dunaliella salina, Botrycoccus braunii, Chlorella vulgaris, Chlorella pyrenoidosa, Serenastrum capricomutum, Scenedesmus auadricauda, Porphyridium cruentum, Scenedesmus acutus, Dunaliella* sp., *Scenedesmus obliquus, Anabaenopsis, Aulosira, Cylindrospermum, Synechoccus* sp., *Synechocystis* sp., *Cyanobacterium aponinum*, and *Tolypothrix* sp..

[0058] Cyanobacteria, also known as blue-green algae, blue-green bacteria, or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. Cyanobacteria can produce metabolites, such as carbohydrates, proteins, lipids and nucleic acids, from CO_2, water, inorganic salts and light. Any Cyanobacteria may be used according to the disclosure. In particular embodiments the Cyanobacteria must be genetically manipulatable, *e.g.*, permissible to the introduction and expression of exogenous (e.g. non-native) genetic material (*e.g.*, exogenous nucleotide sequences).

[0059] Cyanobacteria include both unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types, such as vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation.

[0060] Examples of Cyanobacteria that may be utilized and/or modified according to the methods described herein include *Chroococcales* Cyanobacteria from the genera

Arthrospira, Aphanocapsa, Aphanothece, Chamaesiphon, Chroococcus, Chroogloeocystis, Coelosphaerium, Crocosphaera, Cyanobacterium, Cyanobium, Cyanodictyon, Cyanosarcina, Cyanothece, Dactylococcopsis, Gloecapsa, Gloeothece, Merismopedia, Microcystis, Radiocystis, Rhabdoderma, Snowella, Synychococcus, Synechocystis, Thermosenechococcus, and *Woronichinia; Nostacales* Cyanobacteria from the genera *Anabaena, Anabaenopsis, Aphanizomenon, Aulosira, Calothrix, Coleodesmium, Cyanospira, Cylindrospermosis, Cylindrospermum, Fremyella, Gleotrichia, Microchaete, Nodularia, Nostoc, Rexia, Richelia, Scytonema, Sprirestis*, and *Toypothrix; Oscillatoriales* Cyanobacteria from the genera *Arthrospira, Geitlerinema, Halomicronema, Halospirulina, Katagnymene, Leptolyngbya, Limnothrix, Lyngbya, Microcoleus, Oscillatoria, Phormidium, Planktothricoides, Planktothrix, Plectonema, Pseudoanabaena/Limnothrix, Schizothrix, Symploca, Trichodesmium,* and *Tychonema; Pleurocapsales* Cyanobacteria from the genera *Chroococcidiopsis, Dermocarpa, Dermocarpella, Myxosarcina, Pleurocapsa, Stanieria,* and *Xenococcus; Prochlorophytes* Cyanobacteria from the genera *Prochloron, Prochlorococcus,* and *Prochlorothrix*; and *Stigonematales* Cyanobacteria from the genera *Capsosira, Chlorogeoepsis, Fischerella, Hapalosiphon, Mastigocladopsis, Nostochopsis, Stigonema, Symphyonema, Symphonemopsis, Umezakia*, and *Westiellopsis*. In particular embodiments, the Cyanobacteria is from the genus *Synechococcus*, including *Synechococcus bigranulatus, Synechococcus elongatus, Synechococcus leopoliensis, Synechococcus lividus, Synechococcus nidulans*, and *Synechococcus rubescens.* Cyanobacteria *Thermosynechococcus*, and *Gloeobacter* can also be used.

[0061] More particular embodiments include or utilize *Anabaena* sp. strain PCC 7120, *Synechocystis* sp. strain PCC 6803, *Nostoc muscorum, Nostoc ellipsosporum*, or *Nostoc* sp. strain PCC 7120. In particular embodiments, the Cyanobacteria is *Synechococcus elongatus* sp. strain PCC 7942. Additional examples of Cyanobacteria that may utilized include *Synechococcus* sp. strains WH7803, WH8102, WH8103 (typically modified by conjugation), Baeocyte-forming *Chroococcidiopsis* spp. (typically modified by conjugation/electroporation), non-heterocyst-forming filamentous strains *Planktothrix* sp., *Plectonema boryanum M101* (typically modified by electroporation), Heterocyst-forming *Anabaena* sp. ATCC 29413 (typically modified by conjugation), *Tolypothrix* sp. strain PCC

7601 (typically modified by conjugation/electroporation) and *Nostoc punctiforme* strain ATCC 29133 (typically modified by conjugation/electroporation).

[0062] In particular embodiments, the Cyanobacteria may be, *e.g.*, a marine form of Cyanobacteria or a fresh water form of Cyanobacteria. Examples of marine forms of Cyanobacteria include *Synechococcus* WH8102, *Synechococcus* RCC307, *Synechococcus* NKBG 15041c, and *Trichodesmium*. Examples of fresh water forms of Cyanobacteria include *S. elongatus* PCC 7942, *Synechocystis* PCC6803, *Plectonema boryanum*, *Cyanobacterium aponinum*, and *Anabaena* sp.

[0063] In other embodiments, a modified Cyanobacteria may be capable of growing in brackish or salt water. When using a fresh water form of Cyanobacteria, the overall net cost of their use will depend on both the nutrients required to grow the culture and the price for freshwater. One can foresee freshwater being a limited resource in the future, and in that case it would be more cost effective to find an alternative to freshwater. Two such alternatives include: (1) the use of waste water from treatment plants; and (2) the use of salt or brackish water.

[0064] Salt water in the oceans can range in salinity between 3.1% and 3.8%, the average being 3.5%, and this is mostly, but not entirely, made up of sodium chloride (NaCl) ions. Brackish water, on the other hand, has more salinity than freshwater, but not as much as seawater. Brackish water contains between 0.5% and 3% salinity, and thus includes a large range of salinity regimes and is therefore not precisely defined. Waste water is any water that has undergone human influence. It includes liquid waste released from domestic and commercial properties, industry, and/or agriculture and can encompass a wide range of possible contaminants at varying concentrations.

[0065] There is a broad distribution of Cyanobacteria in the oceans, with *Synechococcus* filling just one niche. Specifically, *Synechococcus* sp. PCC 7002 (formerly known as *Agmenellum quadruplicatum* strain PR-6) grows in brackish water, is unicellular and has an optimal growing temperature of 38°C. While this strain is well suited to grow in conditions of high salt, it will grow slowly in freshwater. In particular embodiments, the disclosure includes the use of a Cyanobacteria PCC 7942, altered in a way that allows for growth in either waste water or salt/brackish water. A *Synechococcus elongatus* PCC 7942 mutant resistant to sodium chloride stress has been described (Bagchi, *et al.*, (2007)

Photosynth Res., 92:87-101), and a genetically modified *S. elongatus* PCC 7942 tolerant of growth in salt water has been described (Waditee, *et al.*, (2002) *PNAS*, 99:4109-4114). Salt water tolerant Cyanobacteria may also be prepared as described in the Examples of U.S. Patent No. 8,394,614. According to the disclosure a salt water tolerant strain is capable of growing in water or media having a salinity in the range of 0.5% to 4.0% salinity, although it is not necessarily capable of growing in all salinities encompassed by this range. In particular embodiments, a salt tolerant strain is capable of growth in water or media having a salinity in the range of 1.0% to 2.0% salinity. In particular embodiments, a salt water tolerant strain is capable of growth in water or media having a salinity in the range of 2.0% to 3.0% salinity.

[0066] Particular mechanisms to modify organisms to utilize non-native bilins rely on inserting exogenous nucleotide sequences into the genome of the selected photosynthetic microorganism. "Exogenous" refers to a nucleotide sequence that does not naturally occur in the particular position of the genome of the wild type photosynthetic microorganism where it is inserted, but is inserted at the particular position by molecular biological techniques. Examples of exogenous nucleotide sequences include vectors, plasmids, and/or man-made nucleic acid constructs.

[0067] As used herein, nucleotide sequences can include genes encoding proteins (e.g., PebA, PebB, PebS, PycA, RpcG, CpcA, CpcB, CpeS, etc.). In relation to genes, this term includes various sequence polymorphisms, mutations, and/or sequence variants. In particular embodiments, the sequence polymorphisms, mutations, and/or sequence variants do not affect the function of the encoded protein. Genes may include not only coding sequences but also non-coding regulatory regions such as promoters, enhancers, and termination regions. The term further can include all introns and other DNA sequences spliced from the mRNA transcript, along with variants resulting from alternative splice sites. Nucleic acid sequences encoding proteins can be DNA or RNA that directs the expression of protein or RNA. These nucleic acid sequences may be a DNA strand sequence that is transcribed into RNA or an RNA sequence that is translated into protein. The nucleic acid sequences include both the full-length nucleic acid sequences as well as non-full-length sequences derived from the full-length protein or RNA. The sequences can also include degenerate codons of the native sequence or

sequences that may be introduced to provide codon preference. Thus, a gene refers to a unit of inheritance that occupies a specific locus on a chromosome and includes transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e., introns, 5' and 3' untranslated sequences).

[0068] A coding sequence is any nucleotide sequence that contributes to the code for the protein product of a gene (e.g., SEQ ID NOs. 4, 5, 7 and 66-70). A non-coding sequence thus refers to any nucleic acid sequence that does not contribute to the code for the protein product of a gene.

[0069] In addition to particular sequences provided, sequences of proteins disclosed herein as well as nucleotide sequences encoding them are available in publicly available databases and publications.

[0070] A "vector" is a nucleotide molecule, (e.g., a DNA molecule) derived, for example, from a plasmid, bacteriophage, yeast or virus, into which a nucleotide sequence (e.g., a gene) can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a photosynthetic microorganism. Autonomously replicating vectors include vectors that exist as extra-chromosomal entities, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. Vectors can also be integrable with the genome of the photosynthetic microorganism. This type of vector is replicated together with the chromosome(s) into which it has been integrated. Such a vector may include specific sequences that allow recombination into a particular, desired site of the host chromosome. Vectors used within the current disclosure can include any mechanism for assuring self-replication. A vector can include a single vector (or plasmid), two or more vectors, three or more vectors, etc. which together contain the total DNA required for expression of a nucleotide sequence of interest to be expressed in the photosynthetic microorganism.

[0071] As indicated, coding sequences to be expressed are operably linked to a promoter; that is they are placed under the regulatory control of a promoter, which then controls the transcription and optionally the translation of the coding sequence. In the construction of heterologous promoter/structural coding sequence combinations, it is generally preferred

to position the promoter at a distance from the coding sequence transcription start site that is approximately the same as the distance between that a promoter and the coding sequence it controls in its natural setting. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a coding sequence to be placed under its control is defined by the positioning of the element in its natural setting; i.e., the genes from which it is derived.

[0072] "Constitutive promoters" are typically active, i.e., promote transcription, under most conditions. "Inducible promoters" are typically active only under certain conditions, such as in the presence of a given molecule factor (e.g., IPTG) or a given environmental condition. In the absence of that condition, inducible promoters typically do not allow significant or measurable levels of transcriptional activity. For example, inducible promoters may be induced according to temperature, pH, a hormone, a metabolite (e.g., lactose, mannitol, an amino acid), light (e.g., wavelength specific), osmotic potential (e.g., salt induced), a heavy metal, or an antibiotic.

[0073] In particular embodiments, the promoter controlling the transcription of the coding sequence of interest can be a Cyanobacterial promoter. The promoter can be endogenous to the modified photosynthetic microorganism or can be a promoter, which was modified in order to increase its efficiency. The promoter can also be a heterologous non-native promoter from a different photosynthetic microorganism species, such as a different Cyanobacterial or bacterial species.

[0074] In particular embodiments, the coding sequence of interest is placed under the transcriptional control of promoters (P) selected from: PaztA (*e.g.*, from *Anabaena* (*Nostoc*) sp. strain PCC 7120); Pc1pB1; PcorT (*e.g.*, from *Synechocystis* sp. PCC6803); PcrhC; PcpcB, (*e.g.*, from Cyanobacteria ABICyano1 (SEQ ID NO: 14)); PcpcBA (*e.g.*, from *Synechocystis* PCC6803); PggpS (*e.g.*, from Cyanobacteria ABICyano1: (SEQ ID NO: 15)); PhliB; PhspA; PhtpG; PisiA; PisiB; PIrtA (*e.g.*, from Cyanobacteria ABICyano1; SEQ ID NO: 16)); PnarB; PnblA (*e.g.*, from Cyanobacteria ABICyano1; (SEQ ID NO: 17)); PnirA; PntcA; PpetE; PpetJ (*e.g.*, from Cyanobacteria ABICyano1; (SEQ ID NO: 18)); PpsbA2; PpsbD; PmrgA (*e.g.*, from Cyanobacteria ABICyano1; (SEQ ID NO: 19)); PnbIA (*e.g.*, from *Nostoc* sp. PCC7120); PnirA (*e.g.*, from Cyanobacteria ABICyano1); PnrsB

(*e.g.*, from *Synechocystis* sp. PCC6803); PnrtA; PntcA; PppsA (*e.g.*, from Cyanobacteria ABICyano1 (SEQ ID NO: 20)); PpsaA; PpsbD; PpstS (*e.g.*, from Cyanobacteria ABICyano1 (SEQ ID NO: 21); PrbcL (*e.g.*, from *Synechocystis* sp. PCC6803); PrbcLS; PrnpA (*e.g.*, from Cyanobacteria ABICyano1 (SEQ ID NO: 22); PrpoA; PrpsL; PsbA2 (*e.g.*, from *Synechocystis* PCC6803); PsigB; PsmtA (*e.g.*, from *Synechococcus* sp. PCC 7002 and *Synechococcus* PCC 7942); and PziaA (*e.g.*, from *Synechocystis* sp. PCC6803). Homologous promoters from other species (*e.g., Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum*) as appropriate can also be used.

[0075] PhspA, Pc1pB1, and PhliB can be induced by heat shock (*e.g.*, raising the growth temperature of the photosynthetic microorganism culture (the culture) from 300°C to 400°C), cold shock (*e.g.*, reducing the growth temperature of the culture from 300°C to 20°C), oxidative stress (*e.g.*, by adding oxidants such as hydrogen peroxide to the culture), or osmotic stress (*e.g.*, by increasing the salinity of the culture). PsigB can be induced by stationary growth, heat shock, and osmotic stress. PntcA and PnblA can be induced by decreasing the concentration of nitrogen in the growth medium and PpsaA and PpsbA2 can be induced by low light or high light conditions. PhtpG can be induced by osmotic stress and heat shock. PcrhC can be induced by cold shock. An increase in copper concentration can be used to induce PpetE, whereas PpetJ is induced by decreasing copper concentration. PaztA, PsmtA, and PziaA can be induced by adding Zn^{2+}. PnrsB can be induced by adding Ni^{2+}. PcorT can be induced by adding cobalt. Additional details of these promoters can be found, for example, in PCT/EP2009/060526.

[0076] Useful constitutive or inducible promoters are also described in, for example: Samartzidou, *et al.*, (1998) *Plant Physiol.*, 117:225-234; Duran, *et al.*, (2004) *J. of Biol. Chem.,* 279:7229-7233; Singh, *et al.*, (2006) *Arch Microbiol.*, 186:273-286; Imamura, *et al.*, (2003) *FEBS Lett.*, 2003; 554:357-362; Imamura, *et al.*, (2006) *J. Biol. Chem.*, 281:2668-2675; Agrawal, *et al.*, (1999) *Biochem. Biophys. Res. Commun.*, 255:47-53; Mohamed, *et al.*, (1989) *Plant Mol. Biol.*, 13:693-700; Muramatsu, *et al.*, (2006) *Plant Cell Physiol.*, 47:878-890; Marin, *et al.*, (2004) *Plant Physiol.*, 136:3290-3300; Marin, *et al.*, (2002) *J. Bacteriol.*, 184:2870-2877; Qi, *et al.*, (2005) *Appl. Environ. Microbiol.*, 71:5678-5684; Maeda *et al.*, (1998) *J. Bacteriol.*; 180:4080-4088; Herranen, *et al.*, (2005) *Plant*

Cell Physiol., 46:1484-1493; Buikema, *et al.*, (2001) *Proc. Natl. Acad. Sci. USA*, 98:2729-2734; Mary, *et al.*, (2004) *Microbiol.*, 150:1271-1281; He, *et al.*, (2001) *J. Biol. Chem.*, 276:306-314; Fang, *et al.*, (2004) *Curr. Microbiol.*, 49:192-198; and Kappell, *et al.*, (2007) *Arch. Microbiol.*, 187:337-342.

[0077] In the case that more than one coding sequence of interest is present, then, for example, the first and second coding sequence can be controlled by one promoter thereby forming a transcriptional operon. Alternatively the first and second coding sequence can be operably linked to different first and second promoters, respectively. When more than one promoter is used, all can be constitutive promoters, all can be inducible promoters, or a combination of constitutive and inducible promoters can be used.

[0078] Expression control can be tightened when mutations are introduced in the TATA-box, the operator sequence and/or the ribosomal binding site (RBS) of the promoter controlling the expression of the coding sequence so that the promoter has at least 90% sequence identity to an endogenous promoter of the modified photosynthetic microorganism. Examples of these approaches are described below in relation to promoters PnirA, PcorT and PsmtA.

[0079] In particular embodiments, PnirA can have the generalized nucleotide sequence of SEQ ID NO: 23 wherein each of the nucleotides n is independently selected from: a, t, c and g and wherein the two (atg)s in the 5'-region of the promoter are the start for NtcB binding sites, gta is the start for the NtcA binding site, ccg denotes the start of the RBS, and the 3'-atg is the start codon for the first recombinant coding sequence transcriptionally controlled by this promoter.

[0080] Another generalized DNA sequence of PnirA includes nucleotide changes in the RBS leading to the generalized DNA sequence of SEQ ID NO: 24. In particular embodiments the modified PnirA can include changes in the operator region (binding site for NtcB and NtcA) and the TATA box leading to the generalized nucleotide sequence of SEQ ID NO: 25. Another variant of PnirA combines changes in the RBS, operator region and the TATA box to form SEQ ID NO: 26.

[0081] Particular embodiments provide the Co^{2+}-inducible PcorT, which has the general nucleotide sequence of SEQ ID NO: 27 wherein each of the nucleotides n is

independently selected from: a, t, c and g and wherein the 5'-cat is the start codon of corR (antisense orientation) and the 3'-atg is the start codon for the first recombinant coding sequence transcriptionally controlled by this promoter. A modified variant of PcorT includes changes in the RBS having SEQ ID NO: 28. Another variant of PcorT includes changes in the TATA box having the general sequence of SEQ ID NO: 29. A third modified PcorT combines the RBS and TATA box modifications into SEQ ID NO: 30.

[0082] Furthermore the Zn^{2+}-inducible PsmtA from *Synechococcus* PCC 7002 can be used having the generalized nucleotide sequence of SEQ ID NO: 31. Changes in the RBS can lead to the following generalized nucleotide sequences of SEQ ID NO: 32 or SEQ ID NO: 33. Again, homologous sequences from other species (*e.g., Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum)* as appropriate may also be used.

[0083] As suggested, particular embodiments include codon optimization. Codons preferred by a particular photosynthetic microorganism can be selected to, for example, increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence. Such nucleotide sequences are typically referred to as "codon-optimized."

[0084] At least some of the nucleotide sequences to be expressed in modified photosynthetic microorganisms can be codon-optimized for optimal expression in a chosen Cyanobacterial strain. The underlying rationale is that the codon usage frequency of highly expressed genes is generally correlated to the host cognate tRNA abundance. (Bulmer, Nature, 1987; 325:728-730). In particular embodiments, the codon optimization is based on the Cyanobacteria ABICyano1 (as well as its close relative species) codon usage frequency (host codon bias), in order to achieve desirable heterologous gene expression (Sharp, *et al.*, (1987); *Nucleic Acids Res.*, 15:1281-1295). In particular embodiments, codon optimization can be based on *Synechococcus elongatus* PCC 7942.

[0085] Codon optimization can be performed with the assistance of publicly available software, such as Gene Designer (DNA 2.0). Additional modifications to minimize unwanted restriction sites, internal Shine-Dalgarno sequences, and other sequences such as internal termination sequences and repeat sequences can also be performed.

These general codon-optimization methods have been shown to result in up to 1,000 fold higher expression of heterologous non-native genes in target organisms (Welch, *et al.*, (2009) PLoS One 4, e7002; and Welch *et al.*, (2009) *J. of the Royal Society*, Interface 6 (Suppl 4):S467-S476.

[0086] In particular embodiments, a gene that encodes one or more proteins (e.g., enzymes, lyases, and/or phycobiloproteins) in a bilin production and function pathway can be placed behind an inducible promoter in a neutral site (e.g., NS1, NS3, NS4) to drive expression of the protein(s).

[0087] In particular embodiments, a gene that has at least 85% sequence identity; 86% sequence identity; 87% sequence identity; 88% sequence identity; 89% sequence identity; 90% sequence identity; 91% sequence identity; 92% sequence identity; 93% sequence identity; 94% sequence identity; 95% sequence identity; 96% sequence identity; 97% sequence identity; 98% sequence identity; or 99% sequence identity to SEQ ID NO: 4, 5, 6, 7, 8, 9, 57, 58, 59, 60, 66, 67, 68, 69 or 70 can be placed behind a promoter to drive expression of PebA, PebB, RpcG, a variant CpcA, CpcB or CpeS respectively. Genes having these sequence identities to other publicly-available relevant gene sequences may also be used (e.g., genes encoding other enzymes, lyases or phycobiloproteins involved in bilin production or function).

[0088] In particular embodiments, a gene that encodes a variant of an enzyme, lyase or phycobiloprotein can be placed behind a promoter to drive expression of the variant.

[0089] Variants of proteins disclosed herein (e.g., PebA, PebB, variant CpcA, CpcB, CpeS, PebS, PycA, RpcG, phycocyanin) include proteins having one or more amino acid additions, deletions, stop positions, or substitutions, as compared to the reference sequence. Variants of proteins have at least 85% sequence identity; 86% sequence identity; 87% sequence identity; 88% sequence identity; 89% sequence identity; 90% sequence identity; 91% sequence identity; 92% sequence identity; 93% sequence identity; 94% sequence identity; 95% sequence identity; 96% sequence identity; 97% sequence identity; 98% sequence identity; or 99% sequence identity to the reference sequence and cause a statistically significant increase in a photosynthetic microorganism's photosynthetic activity in response to a portion of the visible spectrum as compared to a photosynthetic microorganism that has not been modified to utilize a

variant protein to produce a non-native bilin.

[0090] An amino acid substitution can be a conservative or a non-conservative substitution. A "conservative substitution" involves a substitution found in one of the following conservative substitutions groups: Group 1: Alanine (Ala; A), Glycine (Gly; G), Serine (Ser; S), Threonine (Thr; T); Group 2: Aspartic acid (Asp; D), Glutamic acid (Glu; E); Group 3: Asparagine (Asn; N), Glutamine (Gln; Q); Group 4: Arginine (Arg; R), Lysine (Lys; K), Histidine (His; H); Group 5: Isoleucine (Ile; I), Leucine (Leu; L), Methionine (Met; M), Valine (Val; V); and Group 6: Phenylalanine (Phe; F), Tyrosine (Tyr; Y), Tryptophan (Trp; W).

[0091] Additionally, amino acids can be grouped into conservative substitution groups by similar function, chemical structure, or composition (*e.g.*, acidic, basic, aliphatic, aromatic, sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other groups containing amino acids that are considered conservative substitutions for one another include: sulfur-containing: Met and Cys; acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. As indicated, in particular embodiments, conservative substitutions can include substituting Asp56 with Glu, Ser, Thr or Tyr.

[0092] Non-conservative substitutions include those that affect the function of a reference protein in a statistically-significant manner. Non-conservative substitutions include those in which (i) a hydrophilic residue (*e.g.* Ser or Thr) is substituted by a hydrophobic residue (*e.g.* Leu, Ile, Phe, Val, or Ala); (ii) a Cys or Pro is substituted by any other residue; (iii) a residue having an electropositive side chain (*e.g.* Lys, Arg, or His) is substituted by an electronegative residue (*e.g.* Gln or Asp); or (iv) a residue having a bulky side chain (*e.g.* Phe), is substituted by one not having a bulky side chain, (*e.g.* Gly). Additional information is found in Creighton (1984) Proteins, W.H. Freeman and Company.

[0093] In general, non-conservative substitutions will not be made in positions that are conserved across enzymes, lyases or phycobiloproteins (e.g., SEQ ID NOs. 12 and 13 and conserved cysteine residues).

[0094] In particular embodiments, a gene that encodes a protein that has at least 90% sequence identity; 91% sequence identity; 92% sequence identity; 93% sequence identity; 94% sequence identity; 95% sequence identity; 96% sequence identity; 97% sequence identity; 98% sequence identity; 99% sequence identity; or 100% sequence identity to a reference protein variant can be placed behind a promoter to drive expression of proteins in a non-native bilin production and/or function pathway.

[0095] "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Preferred methods to determine sequence identity are designed to give the best match between the sequences tested. Methods to determine sequence identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp, *CABIOS*, 1989; 5:151-153 with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, *et al.*, (1990) *J. Mol. Biol.*, 215:403-410; DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of

the analysis are based on the "default values" of the program referenced. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

[0096] Variants incorporating stop positions can be biologically active fragments. Biologically active fragments have 0.1, 0.5, 1, 2, 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100, 110, 120, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000% or more of the activity of a reference sequence. A reference sequence refers generally to an amino acid sequence or a nucleic acid coding sequence expressing a protein in a non-native bilin synthesis or function pathway activity as described herein. SEQ ID NOs. 1, 2, 3, 61, 62, 63, 64, and 65 provide exemplary reference sequences.

[0097] Insertion (*e.g.*, transformation) of a nucleotide sequence (*e.g.*, a vector) into a photosynthetic microorganism can be achieved using any appropriate method including, for example, natural transformation (*e.g.*, natural DNA uptake; see, *e.g.*, Chung, *et al.*, (1998) *FEMS Microbiol. Lett.*, 164: 353-361; Frigaard, *et al.*, (2004) *Methods Mol. Biol.*,; 274:325-40; Zang, *et al.*, (2007) *J. Microbiol.*, 2007; 45:241-245); conjugation (*e.g.*, bi- or tri-parental mating), transduction, glass bead transformation (see, *e.g.*, Kindle, *et al.*, (1989) *J. Cell Biol.*, 109:2589-601; Feng, *et al.*, (2009) *Mol. Biol. Rep.*, 36:1433-9; U.S. Patent No. 5,661,017), silicon carbide whisker transformation (see, *e.g.*, Dunahay, *et al.*, (1997) *Methods Mol. Biol.*, 62: 503-9), biolistics (see, e.g., Dawson, *et al.*, (1997) *Curr. Microbiol.*, 35: 356-62; Hallmann, *et al.*, (1997) *Proc. Natl. Acad. USA,* 94:7469-7474; Doestch, *et al.*, (2001) *Curr. Genet.*, 39:49-60; Jakobiak, *et al.*, (2004) *Protist,* 155:381-93; Ramesh, *et al.*, (2004) *Methods Mol. Biol.*, 274: 355-307; Tan, *et al.*, (2005) *J. Microbiol.*, 43:361-365; Steinbrenner, *et al.*, (2006) *Appl Environ. Microbiol.*, 72:7477-7484; Kroth, (2007) *Methods Mol. Biol.*, 390:257-267; U.S. Patent No. 5,661,017); electroporation (see, *e.g.*, Kjaerulff, *et al.*, (1994) *Photosynth. Res.*, 41:277-283; Iwai, *et al.*, (2004) *Plant Cell Physiol.*, 45:171-5; Ravindran, *et al.*, (2006) J. *Microbiol. Methods,* 66:174-6; Sun, *et al.*, (2006) *Gene,* 377: 140-149; Wang, *et al.*, (2007) *Appl. Microbiol. Biotechnol.*, 76:651-657; Chaurasia, *et al.*, (2008) *J. Microbiol. Methods,* 73:133-141; Ludwig, *et al.*, (2008) *Appl. Microbiol. Biotechnol.*, 78:729-35), laser-mediated transformation, or incubation with DNA in the presence of or after pre-treatment with any

of poly(amidoamine) dendrimers (see, *e.g.*, Pasupathy, *et al.*, (2008) *J. Biotechnol.,* 3:1078-82), polyethylene glycol (see, *e.g.*, Ohnuma, *et al.*, (2008) *Plant Cell Physiol.*, 49:117-120), cationic lipids (see, *e.g.*, Muradawa, *et al.*, (2008) *J. Biosci. Bioeng.*, 105: 77-80), dextran, calcium phosphate, or calcium chloride (see, *e.g.*, Mendez-Alvarez, *et al.*, (1994) *J. Bacteriol.*, 176:7395-7397), optionally after treatment of the cells with cell wall-degrading enzymes (see, *e.g.*, Perrone, *et al.*, (1998) *Mol. Biol. Cell,* 9:3351-3365).

[0098] In addition, the vector can be modified to allow for integration into a chromosome by adding an appropriate DNA sequence homologous to the target region of the photosynthetic microorganism genome, or through *in vivo* transposition by introducing the mosaic ends (ME) to the vector. Once a plasmid is established in a photosynthetic microorganism, it can be present, for example, at a range of from 1 to many copies per cell.

[0099] Insertion methods described above can be used for introducing nucleotide sequences (*e.g.*, vectors) into Cyanobacterial cells harboring an extracellular polymer layer (EPS). Non-limiting examples for Cyanobacteria with an EPS include several *Nostoc* and *Anabaena* strains, such as *Nostoc commune*, and *Anabanena cylindrica* and several *Cyanothece* sp. strains, such as *Cyanothece* PCC9224, *Cyanothece* CA 3, *Cyanothece* CE 4, *Cyanothece* ET5, *Cyanothece* ET 2, and *Cyanospira capsulata* ATCC 43193. Further examples of Cyanobacteria with an EPS include *Aphanocapsa, Cyanobacterium, Anacystis, Chroococcus, Gloeothece, Microcystis, Synechocystis, Lyngbya, Microcoleus, Oscillatoria, Phormidium, Arthrospira, Anabaena, Cyanospira, Nostoc, Scytonema, Tolypothrix, Chlorogloeopsis, Fischerella*, and *Mastigocladus* (see for example: De Philippis *et al.*, *J. of Applied Phycology*, 2001; 13:293-299; De Philippis, *et al.*, (1998) *FEMS Microbiol. Reviews*, 22:151-175).

[0100] In Cyanobacteria, restriction systems can create barriers to the introduction of exogenous nucleotide sequences. Restriction systems include a restriction enzyme and a specific DNA methyltransferase. Specific methylation of the restriction enzyme recognition sequence protects DNA in the photosynthetic microorganism from degradation by the corresponding restriction enzyme. Knowledge of particular restriction systems within particular bacterial cell types can allow one to protect exogenous nucleotide sequences by methylating it at particular sites to prevent degradation by the

photosynthetic microorganism's restriction system restriction enzyme(s). Thus, an understanding of these restriction systems can be helpful in choosing appropriate transformation protocols for particular bacteria. Particular restriction systems for different Cyanobacterial cells can be found at rebase.neb.com.

[0101] As indicated, nucleotide sequences used herein can include selectable markers to identify genetically modified photosynthetic microorganisms. Selectable markers can be any identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, such as resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the transformation of a nucleotide sequence of interest and/or to identify a genetically modified photosynthetic microorganism that has inherited the nucleotide sequence of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, gentamycin, hygromycin, kanamycin, spectinomycin, streptomycin, fluorescent proteins (*e.g.*, from Promega Corporation, Invitrogen, Clontech, Stratagene, BD Biosciences Pharmingen, Evrogen JSC), and the like.

[0102] Modified photosynthetic microorganisms, including Cyanobacteria, can be cultured or cultivated according to techniques known in the art, such as those described in Acreman, *et al.*, (1994) *J. of Industrial Microbiol. and Biotechnol.*, 13:193-194), in addition to photobioreactor based techniques, such as those described in Nedbal, *et al.*, (2008) *Biotechnol. Bioeng.*, 100:902-10. One example of typical laboratory culture conditions for Cyanobacteria is growth in BG-11 medium (ATCC Medium 616) at 30°C in a vented culture flask with constant agitation and constant illumination at 30-100 µmole photons m^{-2} sec^{-1}.

[0103] Additional media for culturing Cyanobacteria, include Aiba and Ogawa (AO) Medium, Allen and Amon Medium plus Nitrate (ATCC Medium 1142), Antia's (ANT) Medium, Aquil Medium, Ashbey's Nitrogen-free Agar, ASN-III Medium, ASP 2 Medium, ASW Medium (Artificial Seawater and derivatives), ATCC Medium 617 (BG-11 for Marine Blue-Green Algae; Modified ATCC Medium 616 [BG-11 medium]), ATCC Medium 819 (Blue-green Nitrogen-fixing Medium; ATCC Medium 616 [BG-11 medium] without NO_3), ATCC Medium 854 (ATCC Medium 616 [BG-11 medium] with Vitamin B_{12}), ATCC

Medium 1047 (ATCC Medium 957 [MN marine medium] with Vitamin B_{12}), ATCC Medium 1077 (Nitrogen-fixing marine medium; ATCC Medium 957 [MN marine medium] without NO_3), ATCC Medium 1234 (BG-11 Uracil medium; ATCC Medium 616 [BG-11 medium] with uracil), Beggiatoa Medium (ATCC Medium 138), *Beggiatoa* Medium 2 (ATCC Medium 1193), BG-11 Medium for Blue Green Algae (ATCC Medium 616), Blue-Green (BG) Medium, Bold's Basal (BB) Medium, Castenholtz D Medium, Castenholtz D Medium Modified (Halophilic Cyanobacteria), Castenholtz DG Medium, Castenholtz DGN Medium, Castenholtz ND Medium, *Chloroflexus* Broth, *Chloroflexus* Medium (ATCC Medium 920), Chu's #10 Medium (ATCC Medium 341), Chu's #10 Medium Modified, Chu's #11 Medium Modified, DCM Medium, DYIV Medium, E27 Medium, E31 Medium and Derivatives, f/2 Medium, f/2 Medium Derivatives, Fraquil Medium (Freshwater Trace Metal-Buffered Medium), Gorham's Medium for Algae (ATCC Medium 625), h/2 Medium, Jaworski's (JM) Medium, K Medium, L1 Medium and Derivatives, MN Marine Medium (ATCC Medium 957), Plymouth Erdschreiber (PE) Medium, *Prochlorococcus* PC Medium, Proteose Peptone (PP) Medium, Prov Medium, Prov Medium Derivatives, S77 plus Vitamins Medium, S88 plus Vitamins Medium, Saltwater Nutrient Agar (SNA) Medium and Derivatives, SES Medium, SN Medium, Modified SN Medium, SNAX Medium, Soil/Water Biphasic (S/W) Medium and Derivatives, SOT Medium for *Arthrospira (Spirulina)*: ATCC Medium 1679, *Spirulina* (SP) Medium, van Rijn and Cohen (RC) Medium, Walsby's Medium, Yopp Medium, and Z8 Medium, among others.

[0104] Particular embodiments rely on up-regulating or down-regulating a portion of a modified photosynthetic microorganism's genome, in particular embodiments, to reduce or remove the activity of an encoded protein (e.g., a protein in a bilin synthesis or utilization pathway). Down-regulating can be achieved through various mechanisms. Down-regulation can be achieved by, for example, reduction of a gene's copy number, insertion of a foreign set of base pairs into a gene (*e.g.,* into a coding region), deletion of any portion of the gene (*e.g.,* of all or part of a coding region), substitution of base pairs within the gene (*e.g.,* into a coding region), interference with an encoded RNA transcript, the presence of antisense sequences that interfere with transcription or translation of the gene; translation of an incomplete protein; incorrect folding of a protein; expression of an unstable protein; reduced transcription of a gene; incomplete transcription of a gene, or

by any other activity resulting in reduced presence, expression or activity of a protein in the pathway that promotes production or use of a particular bilin.

[0105] Up-regulating can be achieved through, for example, an increase in a gene's copy number, introduction of a strong and/or inducible promoter, mechanisms to prevent degradation of encoding nucleotides or expressed proteins, or other mechanisms.

[0106] As is understood by one of ordinary skill in the art, "up-regulation" and "down-regulation" of gene and protein expression as well as broadened light absorption capability and increased photosynthetic activity can be measured against a relevant control condition including relative to the expression or activity of an unmodified photosynthetic microorganism or a photosynthetic microorganism having a different modification (such as a modification un-related to utilizing a non-native bilin).

[0107] In particular embodiments, broadened light absorption capability means that a modified organism absorbs a wavelength of light in a portion of the visible spectrum that it does not absorb in its non-modified form. In particular embodiments, broadened light absorption capability means that a modified organism absorbs significantly more of a wavelength of light in a portion of the visible spectrum than it does in its non-modified form. In particular embodiments, increased photosynthetic activity means a statistically-significant increase in oxygen evolution at a particular wavelength of light. In particular embodiments, increases in photosynthetic activity can be a statistically-significant increase as compared to a relevant reference level of a relevant control. A measure is not statistically significantly different if the difference is within a level that would be expected to occur based on chance alone. In contrast, a statistically significant difference or increase is one that is greater than what would be expected to occur by chance alone. Statistical significance or lack thereof can be determined by any of various systems and methods used in the art. An example of a commonly used measure of statistical significance is the p-value. The p-value represents the probability of obtaining a given result equivalent to a particular datapoint, where the datapoint is the result of random chance alone. A result is often considered significant (not random chance) at a p-value less than or equal to 0.05.

[0108] The Examples below demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that

many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

EXEMPLARY EMBODIMENTS:

1. A modified photosynthetic microorganism with increased photosynthetic activity as compared to a photosynthetic microorganism of the same species without the modification wherein the increased photosynthetic activity results from the presence of a functional non-native bilin within the modified photosynthetic microorganism.

2. A modified photosynthetic microorganism of embodiment 1 wherein the non-native bilin is phycocyanobilin (PCB), phycoerythrobilin (PEB), phycourobilin (PUB), or phycoviolobilin (PVB).

3. A modified photosynthetic microorganism of embodiment 2 wherein the non-native bilin is PEB or PUB.

4. A modified photosynthetic microorganism of embodiments 1, 2 or 3 wherein the presence of a non-native bilin results in part from the presence of at least one non-native protein.

5. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is an enzyme, lyase, or phycobiloprotein.

6. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is a biliverdin reductase.

7. A modified photosynthetic microorganism of embodiment 6 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

8. A modified photosynthetic microorganism of embodiment 7 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

9. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is RpcG, CpcA, a variant CpcA (e.g., A86K or Y130C), CpcB, and/or CpeS (in particular embodiments RpcG and (i) CpcB and a variant CpcA, or (ii) CpeS).

10. A modified photosynthetic microorganism of embodiment 9 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

11. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

12. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

13. A modified photosynthetic microorganism of embodiment 12 wherein the non-native protein is phycocyanin with a consensus PEB attachment site replaced with a consensus PUB attachment site.

14. A modified photosynthetic microorganism of embodiment 13 wherein the replaced consensus PEB attachment site is SEQ ID NO: 12 and/or the consensus PUB attachment site is SEQ ID NO: 13.

15. A modified photosynthetic microorganism of embodiment 4 wherein the at least one non-native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

16. A modified photosynthetic microorganism of any of embodiments 1-15 wherein the presence of a functional non-native bilin results in part from up-regulation or down-regulation of a portion of the modified photosynthetic microorganism's genome.

17. A modified photosynthetic microorganism of embodiment 16 wherein the up- or down-regulation includes up- or down-regulating the presence or activity of at least one native protein.

18. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is an enzyme, lyase, or phycobiloprotein.

19. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is a biliverdin reductase.

20. A modified photosynthetic microorganism of embodiment 19 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

21. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is RpcG.

22. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

23. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is a native lyase that conjugates native PCB to a specific site on a native phycocyanin.

24. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is a native lyase that is then replaced with a non-native lyase.

25. A modified photosynthetic microorganism of embodiment 24 wherein the native lyase is encoded by a sequence including SEQ ID NO: 10 and the non-native lyase is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

26. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

27. A modified photosynthetic microorganism of embodiment 17 wherein the at least one native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

28. A modified photosynthetic microorganism of any of the proceeding embodiments wherein the genetically-modified photosynthetic microorganism is a Cyanobacteria.

29. A modified photosynthetic microorganism of any of the proceeding embodiments wherein the modified photosynthetic microorganism is a Cyanobacteria selected from

Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.

30. A modified photosynthetic microorganism of any of the proceeding embodiments wherein the increased photosynthetic activity results from broadened light absorption capability and/or a decrease in self-shading.

31. A Cyanobacteria modified to utilize at least one non-native protein involved in bilin production and/or function.

32. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is an enzyme, lyase, or phycobiloprotein.

33. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is a biliverdin reductase.

34. A Cyanobacteria of embodiment 33 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

35. A Cyanobacteria of embodiment 33 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

36. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is RpcG, CpcA, a variant CpcA (e.g., A86K or Y130C), CpcB, and/or CpeS (in particular embodiments, RpcG (i) CpcB and a variant CpcA, or (ii) CpeS).

37. A Cyanobacteria of embodiment 36 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

38. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

39. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

40. A Cyanobacteria of embodiment 39 wherein the non-native protein is phycocyanin with a consensus PEB attachment site replaced with a consensus PUB attachment site.

41. A Cyanobacteria of embodiment 40 wherein the replaced consensus PEB attachment site is SEQ ID NO: 12 and/or the consensus PUB attachment site is SEQ ID NO: 13.

42. A Cyanobacteria of embodiment 31 wherein the at least one non-native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

43. A Cyanobacteria of embodiment 31 further modified to up-regulate or down-regulate a portion of the Cyanobacteria's genome encoding at least one native protein involved in bilin production and/or function.

44. A Cyanobacteria of embodiment 43 wherein the at least one native protein is a biliverdin reductase.

45. A Cyanobacteria of embodiment 44 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

46. A Cyanobacteria of embodiment 45 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

47. A Cyanobacteria of embodiment 43 wherein the at least one native protein is RpcG, CpcA, CpcB, or CpeS.

48. A Cyanobacteria of embodiment 47 wherein

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57

or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

49. A Cyanobacteria of embodiment 43 wherein the at least one native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

50. A Cyanobacteria of embodiment 43 wherein the at least one native protein is a native lyase that conjugates native PCB to a specific site on a native phycocyanin.

51. A Cyanobacteria of embodiment 43 wherein the at least one native protein is a native lyase that is then replaced with a non-native lyase.

52. A Cyanobacteria of embodiment 51 wherein the native lyase is encoded by a sequence including SEQ ID NO: 10 and the non-native lyase is encoded by a sequence including SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

53. A Cyanobacteria of embodiment 43 wherein the at least one native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

54. A Cyanobacteria of embodiment 43 wherein the at least one native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

55. A Cyanobacteria of any of embodiments 42-54 wherein the Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

56. A Cyanobacteria of any of embodiments 42-55 wherein the modification broadens the Cyanobacteria's light absorption capabilities.

57. A Cyanobacteria of any of embodiments 42-56 wherein the modification increases photosynthetic activity.

58. A Cyanobacteria of embodiment 57 wherein the increased photosynthetic activity results from a reduction in self shading.

59. A method to increase photosynthetic activity in a photosynthetic microorganism including modifying the photosynthetic microorganism to utilize a functional non-native

bilin thereby increasing photosynthetic activity.

60. A method of embodiment 59 wherein the modifying leads to utilization (e.g., production) of a functional non-native bilin selected from phycocyanobilin (PCB), phycoerythrobilin (PEB), phycourobilin (PUB), an phycoviolobilin (PVB).

61. A method of embodiment 60 wherein the modifying leads to utilization (e.g., production) of functional non-native PEB or PUB.

62. A method of embodiments 59, 60, or 61 wherein the modifying includes inserting one or more exogenous nucleotide sequences encoding at least one non-native protein involved in bilin synthesis and/or function.

63. A method of embodiment 62 wherein the at least one non-native protein is an enzyme, lyase, or phycobiloprotein.

64. A method of embodiment 62 wherein the at least one non-native protein is a biliverdin reductase.

65. A method of embodiment 64 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

66. A method of embodiment 64 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

67. A method of embodiment 62 wherein the at least one non-native protein is RpcG, CpcA, a variant CpcA (e.g., A86K or Y130C), CpcB and/or CpeS (in particular embodiments, RpcG and (i) CpcB and a variant CpcA, or (ii) CpeS).

68. A method of embodiment 67 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

69. A method of embodiment 62 wherein the at least one non-native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

70. A method of embodiment 62 wherein the at least one non-native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

71. A method of embodiment 62 wherein the non-native protein is phycocyanin with a consensus PEB attachment site replaced with a consensus PUB attachment site.

72. A method of embodiment 71 wherein the replaced consensus PEB attachment site is SEQ ID NO: 12 and/or the consensus PUB attachment site is SEQ ID NO: 13.

73. A method of embodiment 62 wherein the at least one non-native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, variant CpcA (e.g., A86K or Y130C), PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

74. A method of embodiments 59, 60, 61, or 62 wherein the modifying includes or further includes up-regulating or down-regulating one or more endogenous nucleotide sequences encoding at least one native protein involved in bilin synthesis and/or function.

75. A method of embodiment 74 wherein the at least one native protein is a biliverdin reductase.

76. A method of embodiment 75 wherein the biliverdin reductase is PycA, PebA, PebB or PebS.

77. A method of embodiment 75 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

78. A method of embodiment 74 wherein the at least one native protein is RpcG, CpcA, CpcB, and/or CpeS.

79. A method of embodiment 78 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

80. A method of embodiment 62 wherein the at least one native protein is a lyase selected from CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, or PecF.

81. A method of embodiment 74 wherein the at least one native protein is a native lyase that conjugates native PCB to a specific site on a native phycocyanin.

82. A method of embodiment 74 wherein the at least one native protein is a native lyase that is then replaced with a non-native lyase.

83. A method of embodiment 82 wherein the native lyase is encoded by a sequence including SEQ ID NO: 10 and the non-native lyase is encoded by a sequence including SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

84. A method of embodiment 74 wherein the at least one native protein is a phycobiloprotein selected from allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

85. A method of embodiment 74 wherein the at least one native protein is selected from one or more of PycA, PebA, PebB, PebS, RpcG, CpcE, CpeS, CpcF, CpcS, CpcU, CpcT, PecE, PecF allophycocyanin, phycocyanin, CpcA, PecA, CpcB, ApcA, ApcB, ApcD, ApcF, and RpcA.

86. A method of any of embodiments 59-85 wherein the photosynthetic microorganism is a Cyanobacteria.

87. A method of embodiment 86 wherein the Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

88. A method of any of embodiments 59-87 wherein the modifying broadens the Cyanobacteria's light absorption capabilities.

89. A method of any of embodiments 59-88 wherein the modifying increases photosynthetic activity.

90. A method of embodiment 89 wherein the increased photosynthetic activity results

from a reduction in self shading.

91. A method to reduce self-shading including practicing a method of any one of embodiments 59-90.

92. A phycocyanin with a red pigment or a purple pigment.

93. A Cyanobacteria producing a phycocyanin with a red pigment and/or a phycocyanin with a purple pigment.

94. A Cyanobacteria of embodiment 93 wherein the Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

95. A method of producing a Cyanobacteria that produces a phycocyanin with a red pigment and/or a phycocyanin with a purple pigment including selecting a Cyanobacteria that produces native PCB and modifying the Cyanobacteria to produce PEB and/or PUB.

96. A method of embodiment 95 wherein the modifying includes inserting one or more exogenous nucleotide sequences encoding at least one non-native protein involved in PEB and/or PUB synthesis and/or function.

97. A method of embodiment 96 wherein the at least one non-native protein is an enzyme, lyase, or phycobiloprotein.

98. A method of embodiment 96 wherein the at least one non-native protein is a biliverdin reductase.

99. A method of embodiment 98 wherein the biliverdin reductase is PebA or PebB.

100. A method of embodiment 99 wherein the PebA is encoded by a sequence including SEQ ID NO: 4 and/or the PebB is encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

101. A method of embodiment 96 wherein the at least one non-native protein is RpcG, CpcA, a variant CpcA (e.g., A86K or Y130C), CpcB, and/or CpeS (in particular embodiments RpcG and (i) CpcB and a variant CpcA or (ii) CpeS).

102. A method of embodiment 101 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68;

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58; and/or

(v) CpeS is encoded by a sequence including g SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

103. A method of embodiment 96 wherein the non-native protein is phycocyanin with a consensus PEB attachment site replaced with a consensus PUB attachment site.

104. A method of embodiment 103 wherein the replaced consensus PEB attachment site is SEQ ID NO: 12 and/or the consensus PUB attachment site is SEQ ID NO: 13.

105. A method of embodiment 96 wherein the at least one non-native protein is a lyase.

106. A method of embodiments 95 or 96 wherein the modifying includes or further includes deleting or down-regulating one or more endogenous nucleotide sequences encoding at least one native protein involved in PCB, PEB and/or PUB synthesis and/or function.

107. A method of embodiment 106 wherein the at least one native protein is a biliverdin reductase.

108. A method of embodiment 107 wherein the biliverdin reductase is PycA, PebA, or PebB.

109. A method of embodiment 108 wherein the biliverdin reductase is PebA encoded by a sequence including SEQ ID NO: 4 and/or PebB encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

110. A method of embodiment 106 wherein the at least one native protein is a lyase.

111. A method of embodiment 110 wherein the lyase is CpcE.

112. A method of embodiment 110 wherein the lyase is a native lyase that conjugates native PCB to a specific site on a native phycocyanin.

113. A method of embodiment 110 wherein the lyase that is then replaced with a non-native lyase.

114. A method of embodiment 113 wherein the replaced lyase is encoded by a sequence including SEQ ID NO: 10 and the non-native lyase is encoded by a sequence including SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO; 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

115. A method of any of embodiment 95-114 wherein the Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

116. A method of any of embodiments 95-115 wherein the modifying broadens the Cyanobacteria's light absorption capabilities.

117. A method of any of embodiments 95-116 wherein the modifying increases photosynthetic activity.

118. A method of embodiment 117 wherein the increased photosynthetic activity results from a reduction in self shading.

119. A modified Cyanobacteria with increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification wherein the modification results in production of a functioning non-native PEB and the increased photosynthetic activity.

120. A modified Cyanobacteria of embodiment 119 wherein the modification includes insertion of one or more exogenous nucleotide sequences encoding PebA and PebB.

121. A modified Cyanobacteria of embodiment 120 wherein the PebA is encoded by a sequence including SEQ ID NO: 4 and/or the PebB is encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

122. A modified Cyanobacteria of embodiment 119 or 120 wherein a further modification results in production of a functioning non-native PUB and increased photosynthetic activity.

123. A modified Cyanobacteria of embodiment 122 wherein the modification includes insertion of one or more exogenous nucleotide sequences encoding a phycocyanin with a consensus PEB attachment site replaced with a consensus PUB attachment site.

124. A method of embodiment 123 wherein the replaced consensus PEB attachment site is SEQ ID NO: 12 and/or the consensus PUB attachment site is SEQ ID NO: 13.

125. A method of embodiment 122 wherein the modification includes deletion or down-regulation of a native lyase that conjugates native PCB to a specific site on a native phycocyanin.

126. A method of embodiment 125 wherein the modification further includes replacing

the deleted or down-regulated lyase with a non-native lyase.

127. A modified Cyanobacteria of embodiments 122-125 wherein the further modification includes (i) insertion of one or more exogenous nucleotide sequences encoding RpcG and/or CpeS and (ii) deletion or down-regulation of one or more endogenous nucleotide sequences encoding CpcE.

128. A modified Cyanobacteria of embodiment 127 wherein the RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8 and the CpeS is encoded by a sequence including SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

129. A modified Cyanobacteria of embodiment 127 wherein the endogenous nucleotide sequence encoding CpcE includes SEQ ID NO: 10.

130. A modified Cyanobacteria of embodiment 129 wherein deleted or down-regulated SEQ ID NO: 10 is replaced with a sequence including SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

131. A modified Cyanobacteria of embodiment 119 or 120 wherein the further modification includes insertion of one or more exogenous nucleotide sequences encoding RpcG, CpcB, and, a variant CpcA.

132. A modified Cyanobacteria of embodiment 131 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68; and/or

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58.

133. A modified Cyanobacteria of embodiments 119-132 wherein the modified Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

134. A method to increase photosynthetic activity in a Cyanobacteria including inserting one or more exogenous nucleotide sequences encoding PebA and PebB wherein expression of the PebA and PebB results in production of a functioning non-native PEB and the increased photosynthetic activity.

135. A method of embodiment 134 wherein the PebA is encoded by a sequence

including SEQ ID NO: 4 and/or the PebB is encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

136. A method of embodiment 134 or 135 further including inserting one or more exogenous nucleotide sequences encoding RpcG and CpeS and deletion or down-regulation of an endogenous nucleotide sequence encoding CpcE and wherein the additional modifications result in production of a functioning non-native PUB and increased photosynthetic activity.

137. A method of embodiment 134 wherein the RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8, and the CpeS is encoded by a sequence including SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

138. A method of embodiment 136 or 137 wherein the endogenous nucleotide sequence encoding CpcE includes SEQ ID NO: 10.

139. A method of embodiment 138 wherein deleted or down-regulated SEQ ID NO: 10 is replaced with a sequence including SEQ ID NO: 7, or SEQ ID NO: 8, SEQ ID NO: 11, SEQ IE NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 and/or SEQ ID NO: 70.

140. A method of embodiment 134 or 135 further including inserting one or more exogenous nucleotide sequences encoding RpcG, CpcB, and a variant CpcA.

141. A method of embodiment 140 wherein the

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68; and/or

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58.

142. A method of any of embodiments 119-141 wherein the modified Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

143. A method of any of embodiments 119-142 wherein the increased photosynthetic activity results from a broadening of the Cyanobacteria's light absorption capabilities and/or a decrease in self-shading.

144. A Cyanobacteria that naturally produces phycocyanobilin (PCB) wherein the Cyanobacteria is modified to produce functioning non-native PEB and functioning non-native PUB and wherein the Cyanobacteria produces phycocyanin with a red pigment and phycocyanin with a purple pigment.

145. A Cyanobacteria of embodiment 144 that produces functioning non-native PEB due to insertion of one or more exogenous nucleotide sequences encoding PebA and PebB.

146. A Cyanobacteria of embodiment 145 that produces functioning non-native PUB due to insertion one or more exogenous nucleotide sequences encoding PebA, PebB, CpeS and RpcG and deletion or down-regulation of one or more endogenous nucleotide sequences encoding CpcE.

147. A Cyanobacteria of embodiment 145 that produces functioning non-native PUB due to insertion of one or more exogenous nucleotide sequences encoding PebA, PebB, RpcG, CpcB, and a variant CpcA.

148. A method of any of embodiments 145-147 wherein the PebA is encoded by a sequence including SEQ ID NO: 4 and/or the PebB is encoded by a sequence including SEQ ID NO: 5 and/or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

149. A method of embodiment 146 wherein the RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8 and the CpeS is encoded by a sequence including SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

150. A method of any of embodiments 146, 148 or 149 wherein the endogenous nucleotide sequence encoding CpcE includes SEQ ID NO: 10.

151. A method of embodiment 147 or 148 wherein

(i) RpcG is encoded by a sequence including SEQ ID NO: 7 or SEQ ID NO: 8,

(ii) variant CpcA is encoded by a sequence including SEQ ID NO: 66 or SEQ ID NO: 67,

(iii) CpcB is encoded by a sequence including SEQ ID NO: 68; and/or

(iv) variant CpcA and CpcB are encoded by a sequence including SEQ ID NO: 57 or SEQ ID NO: 58.

152. A phycocyanin with a red or purple pigment derived from a cyanobacteria of any

of embodiments 144-151.

153. An embodiment according to any of the preceding embodiments wherein PebA includes SEQ ID NO: 2.

154. An embodiment according to any of the preceding embodiments wherein PebB includes SEQ ID NO: 3.

155. An embodiment according to any of the preceding embodiments wherein RpcG includes SEQ ID NO: 1.

156. An embodiment according to any of the preceding embodiments wherein variant CpcA includes SEQ ID NO: 61.

157. An embodiment according to any of the preceding embodiments wherein Variant CpcA includes SEQ ID NO: 62.

158. An embodiment according to any of the preceding embodiments wherein CpcB includes SEQ ID NO: 63.

159. An embodiment according to any of the preceding embodiments wherein CpeS includes SEQ ID NO: 64.

160. An embodiment according to any of the preceding embodiments wherein CpeS includes SEQ ID NO: 65.

161. A modified Cyanobacteria with increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification wherein the modification includes: (1) insertion of one or more exogenous nucleotide sequences encoding (i) one or more enzymes for synthesis of exogenous bilins, (ii) one or more exogenous bilin lyases, and/or (iii) one or more wild-type and/or variant phycocyanins; and/or (2) (i) insertion of one or more exogenous nucleotide sequences encoding (a) one or more enzymes for synthesis of exogenous bilins, and/or (b) one or more exogenous bilin lyases and (ii) deletion or down-regulation of one or more endogenous nucleotide sequences encoding one or more endogenous bilin lyases.

162. A modified Cyanobacteria with increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification wherein the modification includes: (1) insertion of one or more exogenous nucleotide sequences encoding enzymes for synthesis of exogenous bilins, for an exogenous bilin lyase, and for wild-type and variants of phycocyanin or (2) (i) insertion of one or more exogenous

nucleotide sequences encoding enzymes for synthesis of exogenous bilins, for 2 or more exogenous bilin lyases and (ii) deletion or down-regulation of an endogenous nucleotide sequence encoding an endogenous bilin lyase.

163. An modified Cyanobacteria of embodiment 161 or 162 wherein the enzymes, bilins, bilin lyases, phycocyanins are one or more enzymes, bilins, bilin lyases, phycocyanins disclosed herein.

[0109] As stated previously, CpcE and CpcF often form a heterodimer. Thus, as used herein, and in particular embodiments, deletion or down-regulation of CpcE (including deletion or down-regulation of an endogenous nucleotide sequence encoding CpcE), can include deletion or down-regulation of CpcE itself and/or deletion or down-regulation of CpcF, as deletion or down-regulation of either or both deletes or down-regulates CpcE activity when that activity is based on heterodimer formation.

[0110] Detailed Experimental Methods. Organisms and culture conditions. Cyanobacteria strains used in the experiments of the disclosure were either wild type *Syn* 7942 or mutant strains derived from *Syn* 7942. All strains were grown photoautotrophically under fluorescent light (120 $\mu E \times m^{-2} \times s^{-1}$) in 3×BG-11 medium, 10 mM sodium phosphate (pH 7.2), at 30º C, 1% CO_2, and continuous agitation on an orbital shaker (200 rpm).

[0111] Bilin absorption spectra. Because the detection of bilins can be hindered in whole cell absorption spectra by the presence of other pigments such as carotenoids, water soluble cell free lysate was prepared. Cultures were harvested by centrifugation (3,000×g), resuspended in 150 µl of buffer (consisting of 20mM HEPES + 10 mM EDTA + 100 mM DTT + 100 mM Na_2CO_3 at pH 7), added to glass beads (100 µm diameter), and placed in the bead beater for 3 min (30 seconds on, rest 30 seconds). Liquid was then transferred to a 1.7 ml centrifuge tube and centrifuged for 20 min at 14,000 rpm. The absorption spectra of the resultant cell and membrane free supernatant were taken with a SpectraMax M5 spectrometer.

[0112] Further fractionation of the cell free lysate into allophycocyanin or phycocyanin was accomplished by ammonium sulfate precipitation. Allophycocyanin precipitated at approximately 40% ammonium sulfate and phycocyanin precipitated at 50% ammonium sulfate. Following fractionation and precipitation, allophycocyanin or phycocyanin were resuspended in 10 mM HEPES (pH 7) and absorption spectra were taken as with the

SpectraMax M5 spectrometer.

[0113] Action Spectra. Action spectra in which different wavelengths of light were tested for their ability to stimulate oxygen evolution in whole cells of *Syn* 7942 were taken using a Clark style O_2 electrode and LED light sources. LED lighting was provided using a variable output device that could be adjusted from 20-1000 $\mu E \times m^{-2} \times s^{-1}$ and light could be supplied as 405 nm, 450 nm, 505 nm, 520 nm, 590 nm, 650 nm, or 710 nm wavelength light. In addition white LED light (4,000 K) could also be supplied by the light source. The LED light source was produced by Reliance Laboratories LLC, 1240 West Sims Way #137, Port Townsend, WA 98368.For whole cell action spectra, cultures were harvested by centrifugation (14,000 rpm) and resuspended in fresh BG-11 medium with 20 mM Sodium bicarbonate and 10 mM HEPES (pH 7.2). To remove some oxygen from resuspended cultures, cultures were flushed for 10 seconds with either Argon or Nitrogen gas. Resuspendened cells were then immediately placed into the Clark electrode chamber, allowed to equilibrate for 2 min in the dark with constant stirring, and then supplied light at the wavelength and intensity indicated in the figures.

[0114] SDS PAGE and Zinc Acetate staining. Three ml of culture at OD_{750} of 1 were harvested by centrifugation (3,000×g), resuspended in 150 µl of buffer (consisting of 20mM HEPES + 10 mM EDTA + 100 mM DTT + 100 mM Na_2CO_3 at pH 7). Samples were added to glass beads, broken in a bead beater, and membranes were separated from soluble cell lysate by centrifugation at 14,000 rpm for 20 min. 80 µL of the water soluble supernatant was added to 54 µL of a solution of 30% sucrose and 5% SDS. A small hole was then placed in each sample tube with a needle, the samples were boiled for 60 seconds, cooled on ice for 2 minutes, and loaded onto 4-20% gradient of polyacrylamide gel. Gels were run at 165 volts for 1 hour, and phycobiliproteins could be visualized based upon their color. Gels were then stained with 20 mM zinc acetate for 20 min and visualized with a blue light source (470 nm wavelength light) and an amber filter. Bilins could then be identified based upon fluorescence.

[0115] Detection of phycobilins by LC/MS. The partially purified PCB was solubilized in 1ml of MeOH. The blue/green solution was analyzed by LC/MS to identify major conformations of PCB and degradation products using a Waters 2695 separation module with a Waters 2998 Photodiode array (PDA), Waters 2424 ELSD, and a Micromass ZQ.

Electro spray source (ESI+) 150-2000, multiple 25ul injections were employed for analysis. The sample was separated on a Phenomenex Gemini C-18: 250mm x 4.6mm; 4.6um column at 25C. A linear gradient of Water, 0.1% trifluoroacetic acid (A) and acetonitrile, 0.1% trifluoroacetic acid (B) as follows: T=0 min (A=25% B=75%), T=30 min (A=75% B=25%). Metabolites were monitored by UV/Vis from 215nm-700nm and by mass from 150 to 2000 amu.

[0116] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." As used herein, the transition term "comprise" or "comprises" means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. As used herein, a material effect would cause a statistically-significant reduction in the broadening of light absorbance capabilities.

[0117] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, *i.e.* denoting somewhat more or somewhat less than the stated value or range, to within a range of ±20% of the stated value; ±19% of the stated value; ±18% of the stated value;

±17% of the stated value; ±16% of the stated value; ±15% of the stated value; ±14% of the stated value; ±13% of the stated value; ±12% of the stated value; ±11% of the stated value; ±10% of the stated value; ±9% of the stated value; ±8% of the stated value; ±7% of the stated value; ±6% of the stated value; ±5% of the stated value; ±4% of the stated value; ±3% of the stated value; ±2% of the stated value; or ±1% of the stated value.

[0118] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0119] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0120] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0121] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0122] Furthermore, if references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein), each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0123] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0124] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0125] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any

construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

CLAIMS

WHAT IS CLAIMED IS:

1. A modified Cyanobacteria comprising: (1) insertion of one or more exogenous nucleotide sequences encoding PebA, PebB, RpcG, CpcB, and a variant CpcA; or (2) (i) insertion of one or more exogenous nucleotide sequences encoding PebA, PebB, RpcG and CpeS and (ii) deletion of an endogenous nucleotide sequence encoding CpcE, the modified Caynaobacteria having increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification.

2. A modified Cyanobacteria of claim 1 wherein the PebA comprises SEQ ID NO: 2.

3. A modified Cyanobacteria of claim 1 wherein the PebB comprises SEQ ID NO: 3.

4. A modified Cyanobacteria of claim 1 wherein the PebA is encoded by a sequence comprising SEQ ID NO: 4, the PebB is encoded by a sequence comprising SEQ ID NO: 5, or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

5. A modified Cyanobacteria of claim 1 wherein the RpcG comprises SEQ ID NO: 1.

6. A modified Cyanobacteria of claim 1 wherein the RpcG is encoded by a sequence comprising SEQ ID NO: 7.

7. A modified Cyanobacteria of claim 1 wherein the variant CpcA comprises SEQ ID NO: 61 or SEQ ID NO: 62.

8. A modified Cyanobacteria of claim 1 wherein the variant CpcA is encoded by a sequence comprising SEQ ID NO: 66 or SEQ ID NO: 67.

9. A modified Cyanobacteria of claim 1 wherein the CpcB comprises SEQ ID NO: 63.

10. A modified Cyanobacteria of claim 1 wherein the CpcB is encoded by a sequence comprising SEQ ID NO: 68.

11. A modified Cyanobacteria of claim 1 wherein the CpeS comprises SEQ ID NO: 64 or SEQ ID NO: 65.

12. A modified Cyanobacteria of claim 1 wherein the CpeS is encoded by a sequence comprising SEQ ID NO: 69 or SEQ ID NO: 70.

13. A modified Cyanobacteria of claim 1 wherein the endogenous nucleotide sequence encoding CpcE comprises SEQ ID NO: 10.

14. A modified Cyanobacteria of claim 13 wherein deleted SEQ ID NO: 10 is replaced with a sequence comprising SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO:

59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

15. A modified Cyanobacteria of claim 1 wherein the modified Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

16. A modified Cyanobacteria with increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification wherein the modification results in utilization of a functioning non-native PEB and the increased photosynthetic activity.

17. A modified Cyanobacteria of claim 16 wherein the modification results in expression of a PebA protein and a PebB protein.

18. A modified Cyanobacteria of claim 17 wherein the PebA comprises SEQ ID NO: 2.

19. A modified Cyanobacteria of claim 17 wherein the PebB comprises SEQ ID NO: 3.

20. A modified Cyanobacteria of claim 16 wherein the modification comprises insertion of one or more exogenous nucleotide sequence encoding PebA and PebB.

21. A modified Cyanobacteria of claim 17 wherein the PebA is encoded by a sequence comprising SEQ ID NO: 4, the PebB is encoded by a sequence comprising SEQ ID NO: 5, or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

22. A modified Cyanobacteria of claim 16 wherein a further modification results in utilization of a functioning non-native PUB and increased photosynthetic activity.

23. A modified Cyanobacteria of claim 23 wherein the further modification results in expression of an RpcG protein and (i) a CpcB protein and a variant CpcA protein or (ii) a CpeS protein.

24. A modified Cyanobacteria of claim 23 wherein the RpcG protein comprises SEQ ID NO: 1.

25. A modified Cyanobacteria of claim 23 wherein the RpcG protein is encoded by a sequence comprising SEQ ID NO: 7.

26. A modified Cyanobacteria of claim 23 wherein the further modification results in expression of a CpcB protein.

27. A modified Cyanobacteria of claim 26 wherein the CpcB protein comprises SEQ

ID NO: 63.

28. A modified Cyanobacteria of claim 26 wherein the CpcB protein is encoded by a sequence comprising SEQ ID NO: 68.

29. A modified Cyanobacteria of claim 26 wherein the further modification results in expression of a variant CpcA protein.

30. A modified Cyanobacteria of claim 29 wherein the variant CpcA protein comprises SEQ ID NO: 61 or SEQ ID NO: 62.

31. A modified Cyanobacteria of claim 29 wherein the variant CpcA protein is encoded by a sequence comprising SEQ ID NO: 66 or SEQ ID NO: 67.

32. A modified Cyanobacteria of claim 23 wherein the further modification results in expression of a CpeS protein.

33. A modified Cyanobacteria of claim 32 wherein the CpeS protein comprises SEQ ID NO: 64 or SEQ ID NO: 65.

34. A modified Cyanobacteria of claim 32 wherein the CpeS protein is encoded by a sequence comprising SEQ ID NO: 69 or SEQ ID NO: 70.

35. A modified Cyanobacteria of claim 22 wherein the further modification comprises down-regulation of an endogenous nucleotide sequence encoding CpcE.

36. A modified Cyanobacteria of claim 35 wherein the endogenous nucleotide sequence encoding CpcE comprises SEQ ID NO: 10.

37. A modified Cyanobacteria of claim 36 wherein down-regulation includes deletion of SEQ ID NO: 10 accompanied by replacement with a sequence comprising SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

38. A modified Cyanobacteria of claim 16 or 22 wherein the modified Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

39. A method to increase photosynthetic activity in a Cyanobacteria comprising inserting one or more exogenous nucleotide sequences encoding PebA and PebB wherein expression of the PebA and PebB results in production of a functioning non-native PEB and the increased photosynthetic activity.

40. A method of claim 39 wherein the PebA is encoded by a sequence comprising

SEQ ID NO: 4, the PebB is encoded by a sequence comprising SEQ ID NO: 5, or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9.

41. A method of claim 39 wherein the modification further comprises inserting one or more exogenous nucleotide sequences encoding RpcG, CpcB, and a variant CpcA wherein the further modifications result in production of a functioning non-native PUB and increased photosynthetic activity.

42. A method of claim 41 wherein the RpcG is encoded by a sequence comprising SEQ ID NO: 7.

43. A method of claim 41 wherein the CpcB is encoded by a sequence comprising SEQ ID NO: 68.

44. A method of claim 41 wherein the variant CpcA is encoded by a sequence comprising SEQ ID NO: 66 or a sequence comprising SEQ ID NO: 67.

45. A method of claim 39 wherein the modification further comprises inserting one or more exogenous nucleotide sequences encoding RpcG and CpeS and deleting one or more endogenous nucleotide sequences encoding CpcE wherein the further modifications result in production of a functioning non-native PUB and increased photosynthetic activity.

46. A method of claim 45 wherein the RpcG is encoded by a sequence comprising SEQ ID NO: 7.

47. A method of claim 45 wherein the CpeS is encoded by a sequence comprising SEQ ID NO: 69 or SEQ ID NO: 70.

48. A method of claim 45 wherein the endogenous nucleotide sequence encoding CpcE comprises SEQ ID NO: 10.

49. A method of claim 48 wherein deleted SEQ ID NO: 10 is replaced with a sequence comprising SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 69 or SEQ ID NO: 70.

50. A method of claim 39, 41, or 45 wherein the modified Cyanobacteria is selected from *Synechococcus elongatus, Arthrospira maxima, Arthrospira platensis, and Cyanobacterium aponinum.*

51. A method of claim 39, 41, or 45 wherein the increased photosynthetic activity results from a broadening of the Cyanobacteria's light absorption capabilities and/or a

decrease in self-shading.

52. A Cyanobacteria that naturally produces phycocyanobilin (PCB) wherein the Cyanobacteria is modified to produce functioning non-native PEB and functioning non-native PUB and wherein the Cyanobacteria produces phycocyanin with a red pigment and phycocyanin with a purple pigment.

53. A Cyanobacteria of claim 52 modified to express PebA, PebB, and RpcG in combination with (i) CpcB and a variant CpcA or (ii) CpeS in the presence of down-regulated endogenous CpcE.

54. A Cyanobacteria of claim 53 wherein the PebA comprises SEQ ID NO: 2.

55. A Cyanobacteria of claim 53 wherein the PebB comprises SEQ ID NO: 3.

56. A Cyanobacteria of claim 53 wherein the PebA is encoded by a sequence comprising SEQ ID NO: 4, the PebB is encoded by a sequence comprising SEQ ID NO: 5, or PebA and PebB encoded by a sequence including SEQ ID NO: 6 or SEQ ID NO: 9

57. A Cyanobacteria of claim 53 wherein the RpcG comprises SEQ ID NO: 1.

58. A Cyanobacteria of claim 53 wherein the RpcG is encoded by a sequence comprising SEQ ID NO: 7.

59. A Cyanobacteria of claim 53 wherein the CpcB comprises SEQ ID NO: 63.

60. A Cyanobacteria of claim 53 wherein the CpcB is encoded by a sequence comprising SEQ ID NO: 68.

61. A Cyanobacteria of claim 53 wherein the variant CpcA comprises SEQ ID NO: 61 or SEQ ID NO: 62.

62. A Cyanobacteria of claim 53 wherein the variant CpcA is encoded by a sequence comprising SEQ ID NO: 66 or SEQ ID NO: 67.

63. A Cyanobacteria of claim 53 wherein the CpeS comprises SEQ ID NO: 64 or SEQ ID NO: 65.

64. A Cyanobacteria of claim 53 wherein the CpeS is encoded by a sequence comprising SEQ ID NO: 69 or SEQ ID NO: 70.

65. A Cyanobacteria of claim 53 wherein the endogenous CpcE is down-regulated due to deletion of a sequence comprising SEQ ID NO: 10.

66. A Cyanobacteria of claim 65 wherein absent SEQ ID NO: 10 is replaced with a sequence comprising SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 59,

SEQ ID NO: 60, SEQ ID NO: 69, or SEQ ID NO: 70.

67. A phycocyanin with a red or purple pigment derived from a cyanobacteria of claim 53.

FIG. 1

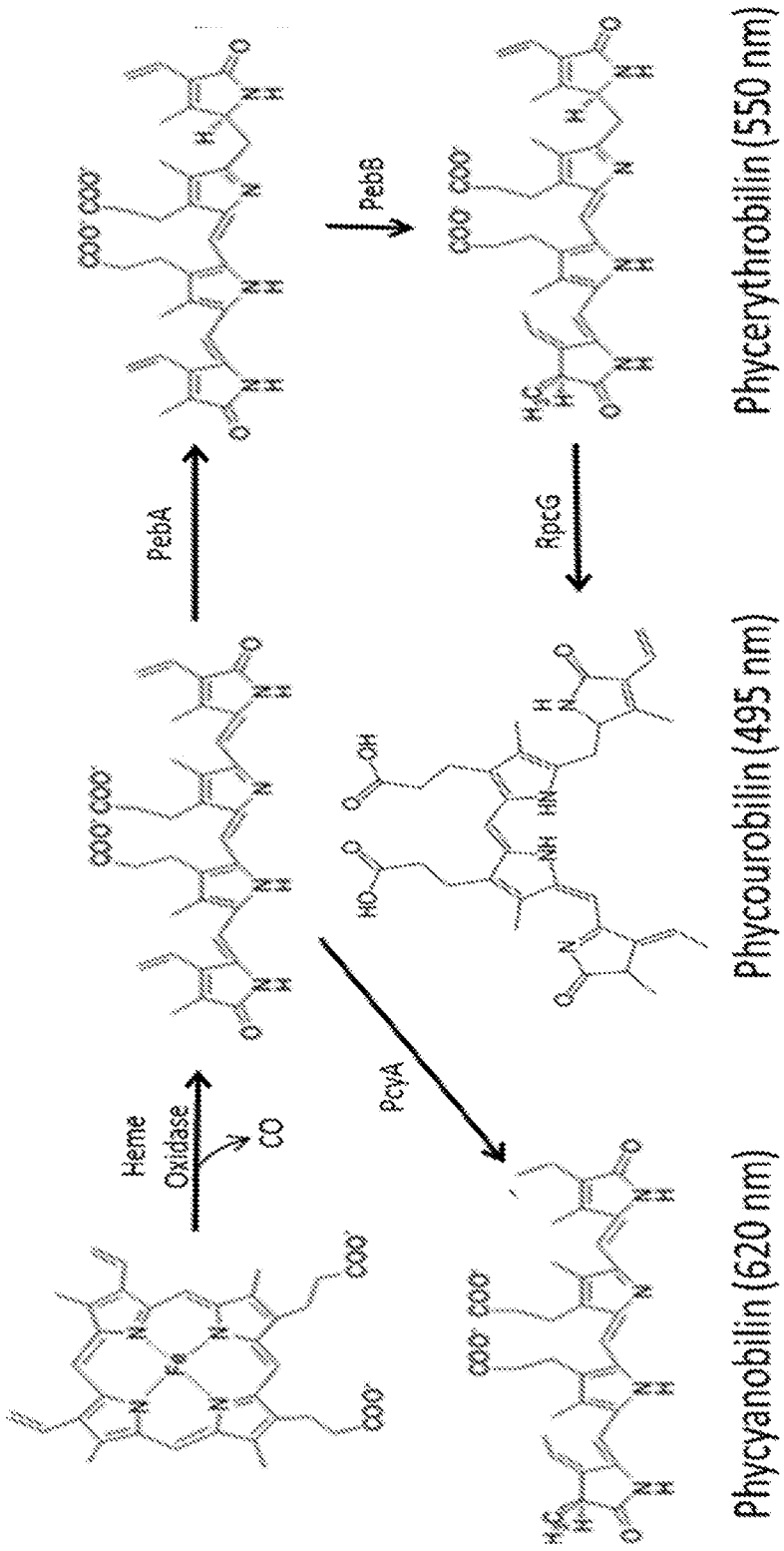
Heme
Oxidase
CO
PebA
PebB
RpcG
PcyA
Phycyanobilin (620 nm)
Phycourobilin (495 nm)
Phycerythrobilin (550 nm)

FIG. 2

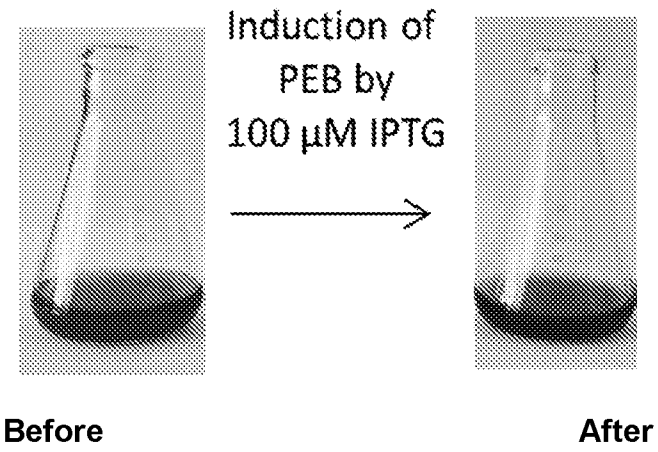
Induction of
PEB by
100 µM IPTG
Before
After

FIG. 3

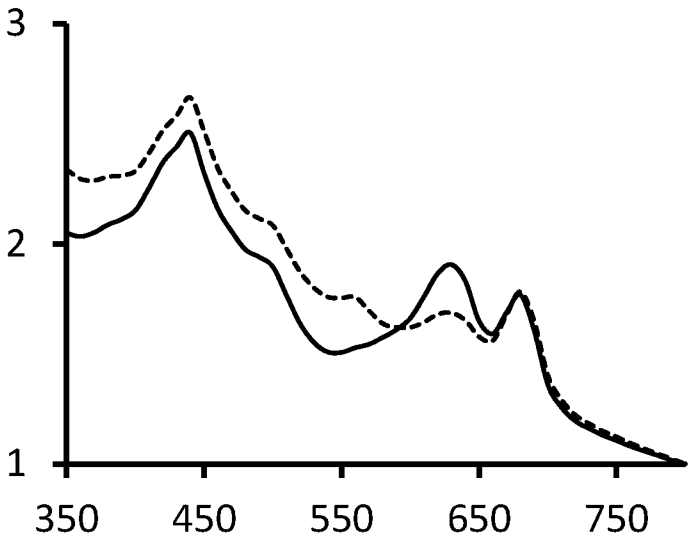
3
2
1
350
450
550
650
750

FIG. 4

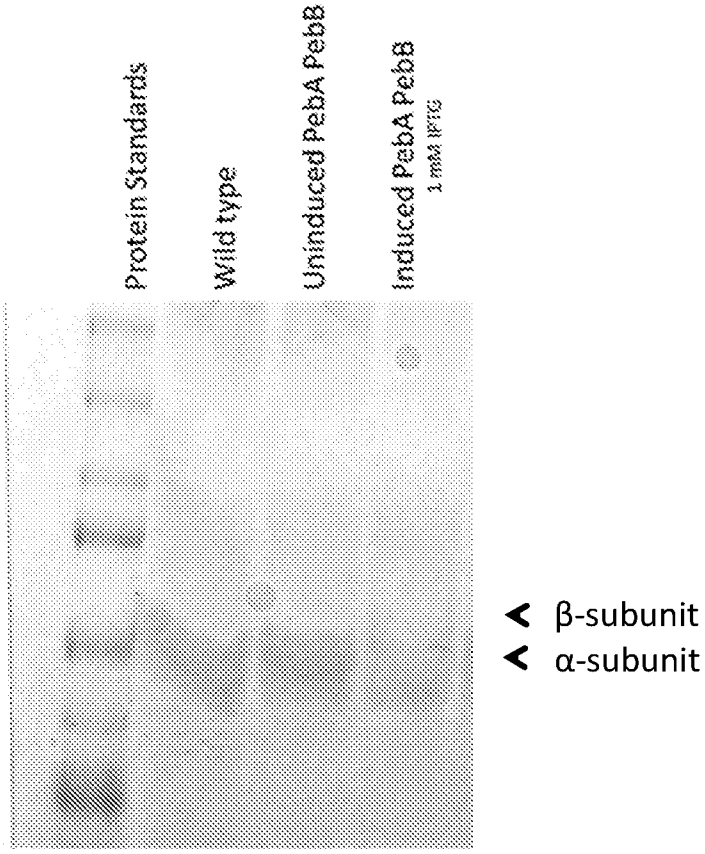
Protein Standards
Wild type
Uninduced PebA PebB
Induced PebA PebB
1 mM IPTG
β-subunit
α-subunit

FIG. 5A

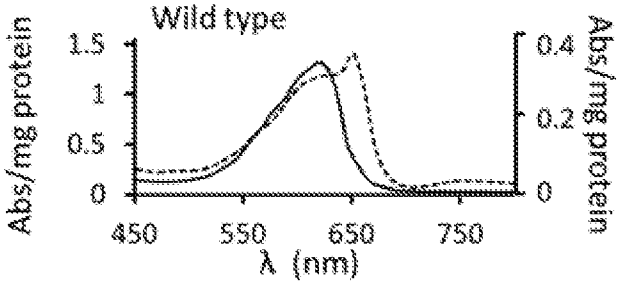

FIG. 5B

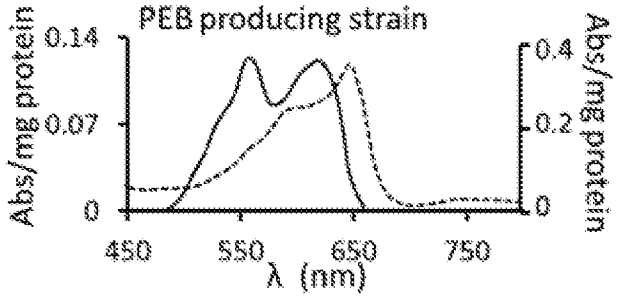

FIG. 5C

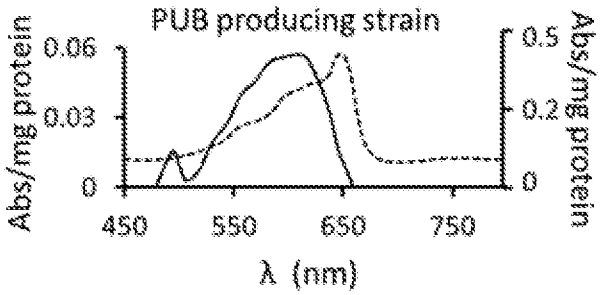

FIG. 5D

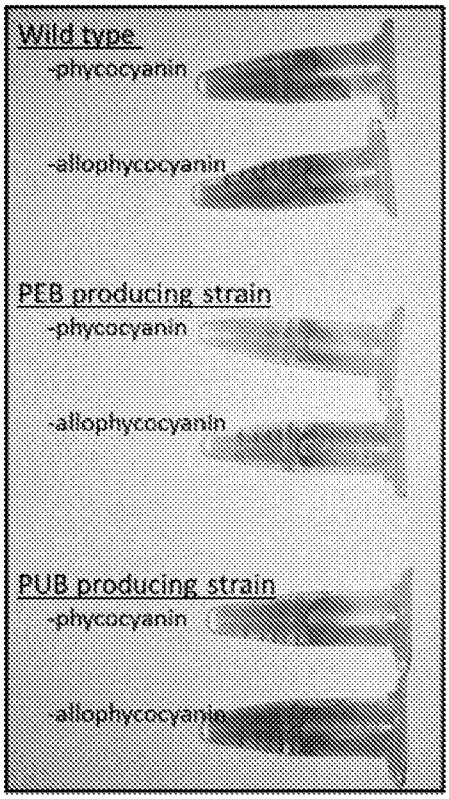
Wild type
-phycocyanin
-allophycocyanin
PEB producing strain
-phycocyanin
-allophycocyanin
PUB producing strain
-phycocyanin
-allophycocyanin

FIG. 5E

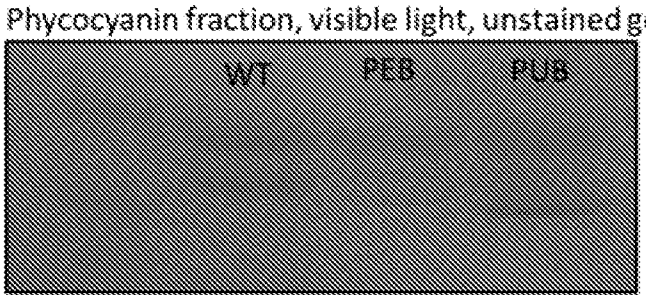
Phycocyanin fraction, visible light, unstained gel
WT
PEB
PUB

FIG. 5F

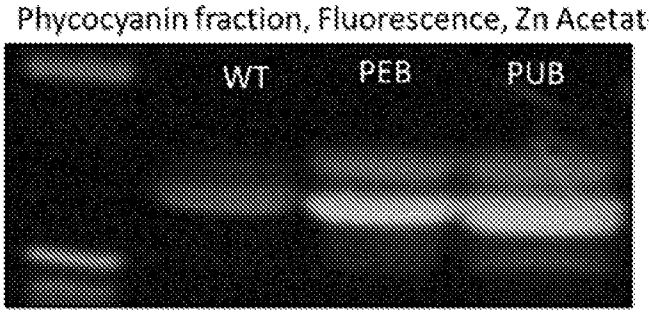
Phycocyanin fraction, Fluorescence, Zn Acetate
WT
PEB
PUB

FIG. 5G

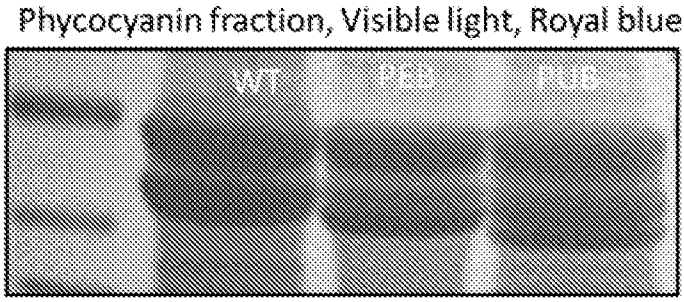
Phycocyanin fraction, Visible light, Royal blue
WT
PEB
PUB

FIG. 6A

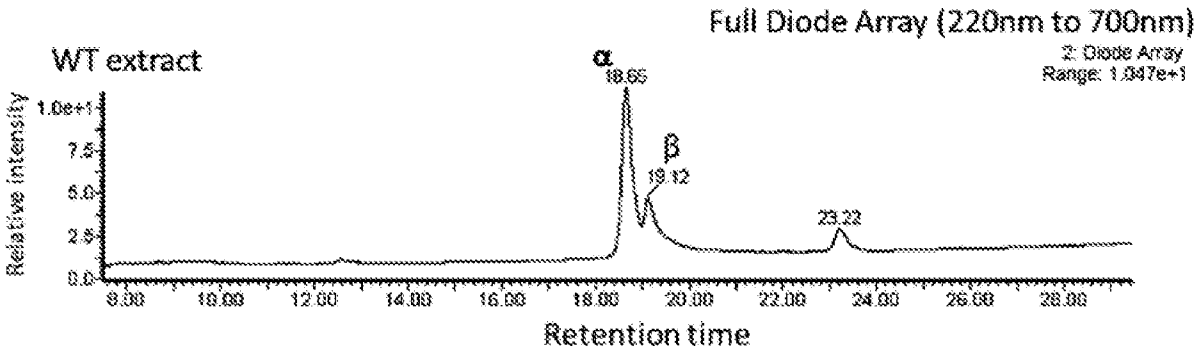

FIG. 6B

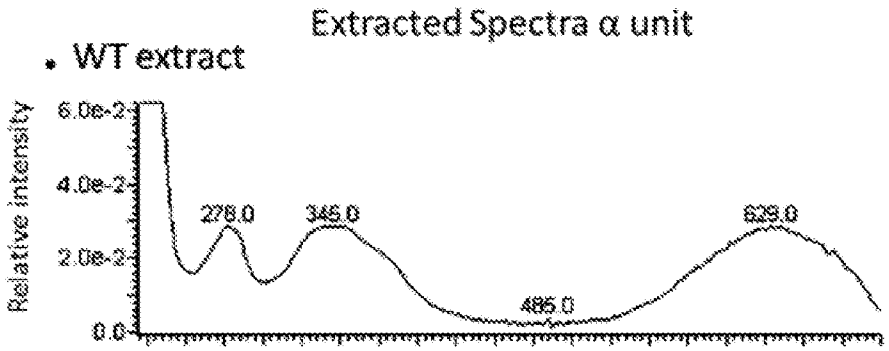

FIG. 6C

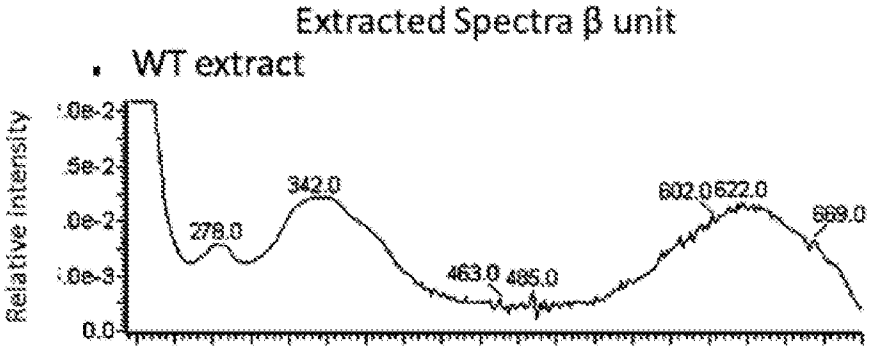

FIG. 6D

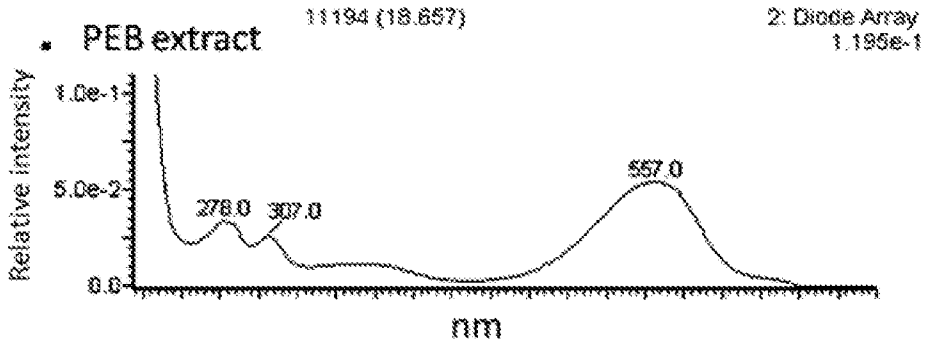
PEB extract
11194 (18.657)
2: Diode Array
1.195e-1
Relative intensity
1.0e-1
5.0e-2
0.0
278.0
307.0
557.0
nm

FIG. 6E

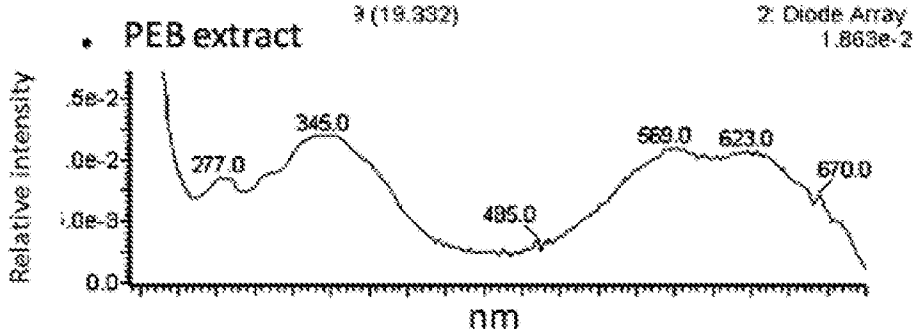
PEB extract
3 (19.332)
2: Diode Array
1.863e-2
Relative intensity
5e-2
0e-2
0e-3
0.0
277.0
346.0
495.0
569.0
623.0
670.0
nm

FIG. 7A

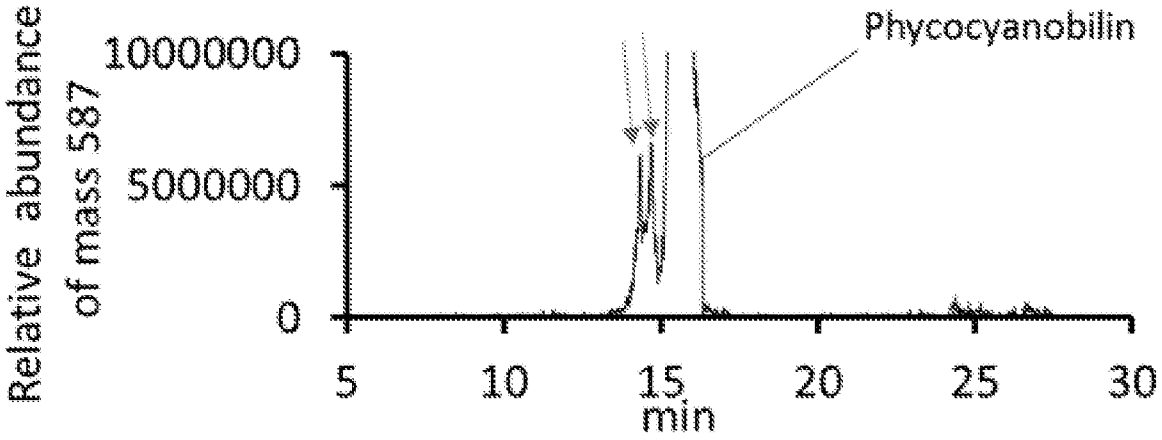
Relative abundance of mass 587
10000000
5000000
0
5
10
15
20
25
30
min
Phycocyanobilin

FIG. 7B

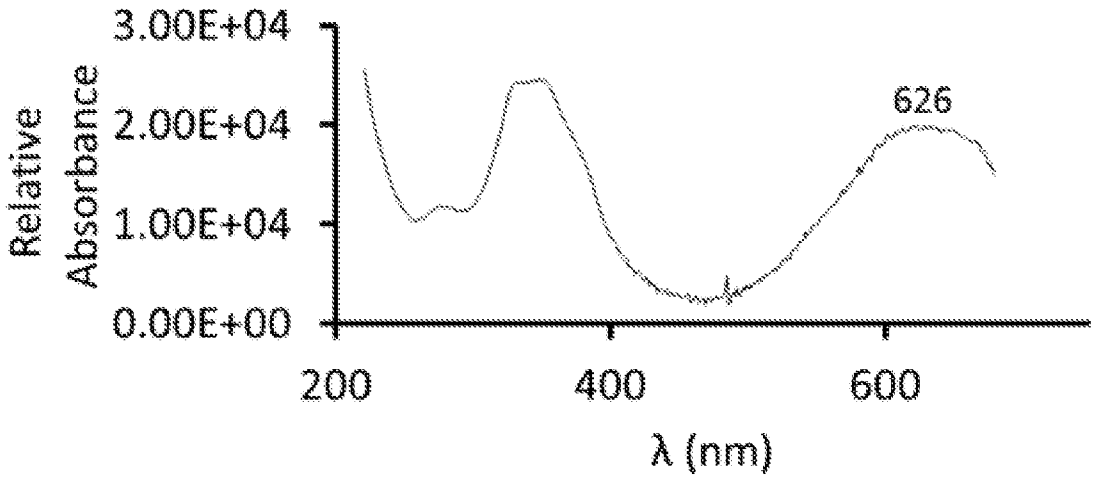
Relative Absorbance
3.00E+04
2.00E+04
1.00E+04
0.00E+00
200
400
600
λ (nm)
626

FIG. 7C

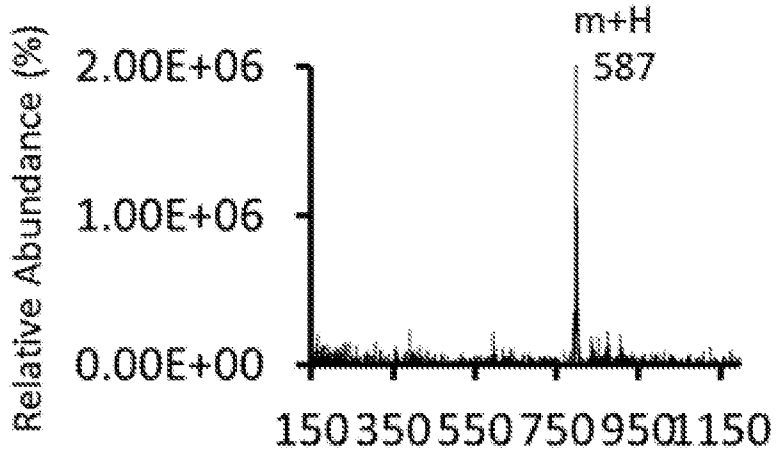
m+H
587
Relative Abundance (%)
2.00E+06
1.00E+06
0.00E+00
150 350 550 750 950 1150

FIG. 7D

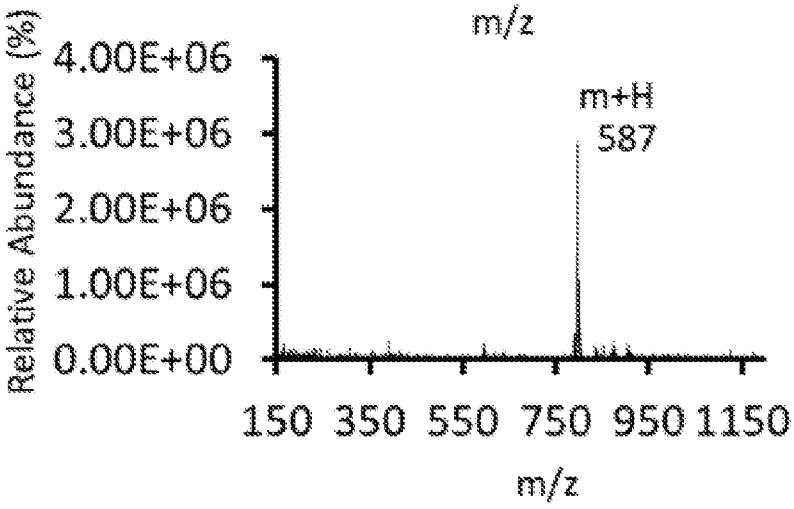
m/z
m+H
587
Relative Abundance (%)
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
150 350 550 750 950 1150
m/z

FIG. 8A

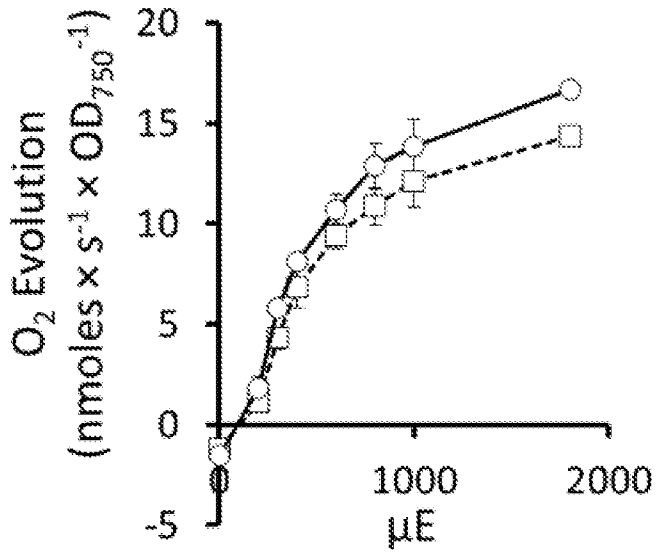
O_2 Evolution
(nmoles × s^{-1} × OD_{750}^{-1})
20
15
10
5
0
-5
0
1000
2000
µE

FIG. 8B

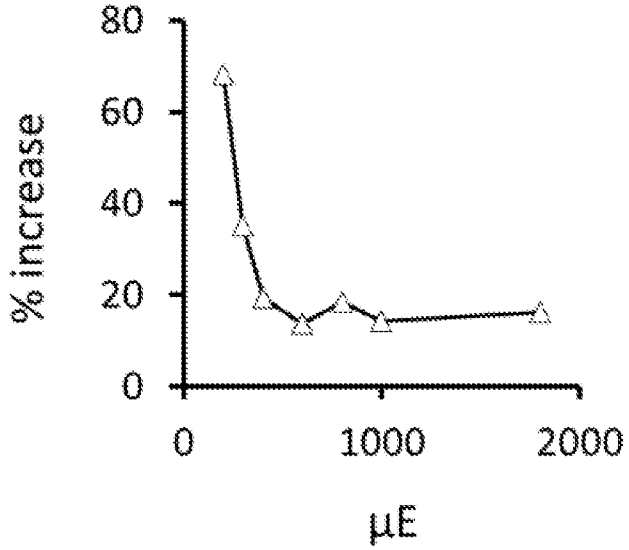
% increase
80
60
40
20
0
0
1000
2000
µE

FIG. 9A

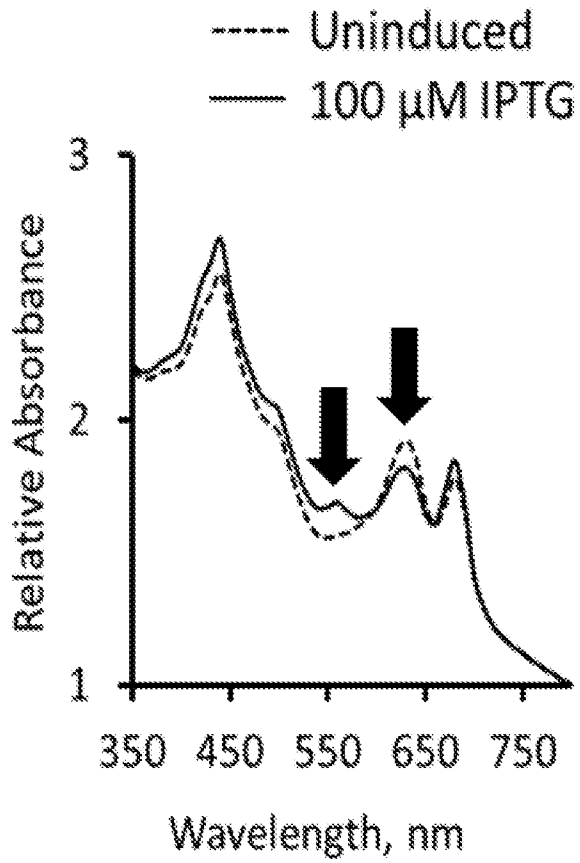
Uninduced
100 µM IPTG
Relative Absorbance
3
2
1
350
450
550
650
750
Wavelength, nm

FIG. 9B

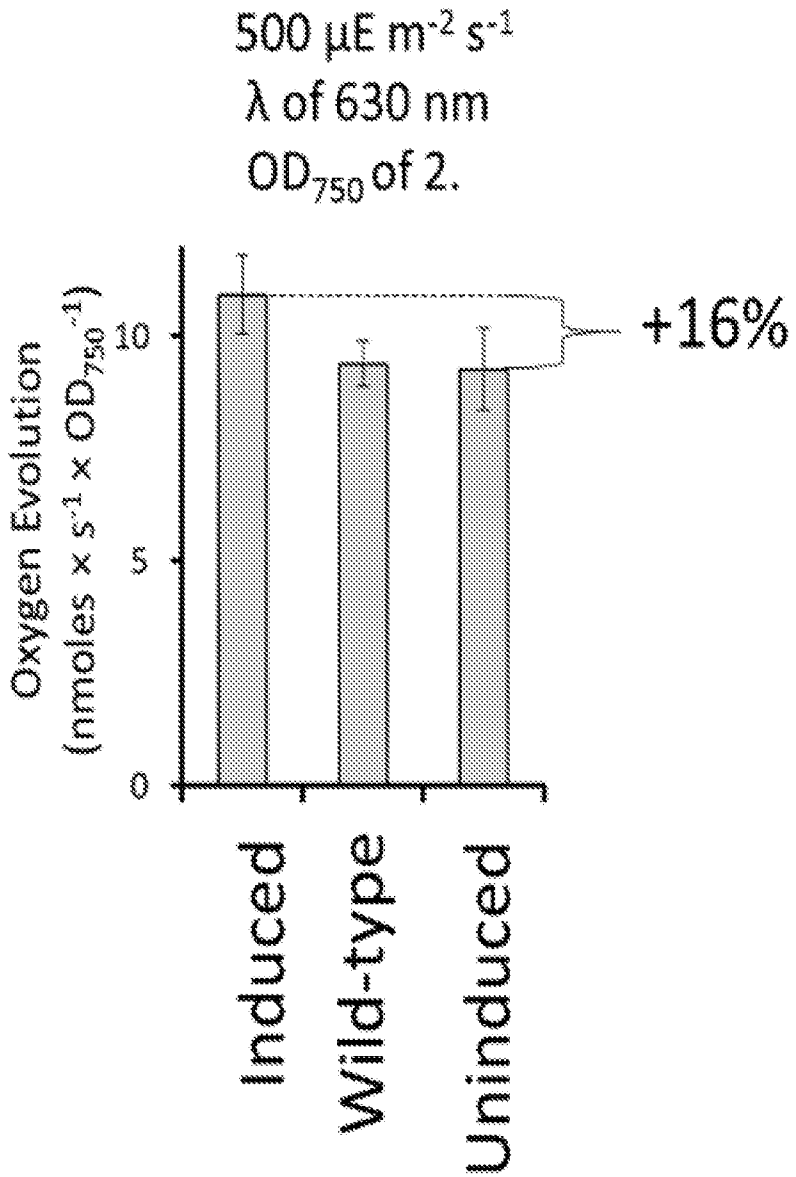
500 µE m-2 s-1
λ of 630 nm
OD750 of 2.
Oxygen Evolution
(nmoles × s-1 × OD750-1)
10
5
0
+16%
Induced
Wild-type
Uninduced

FIG. 9C

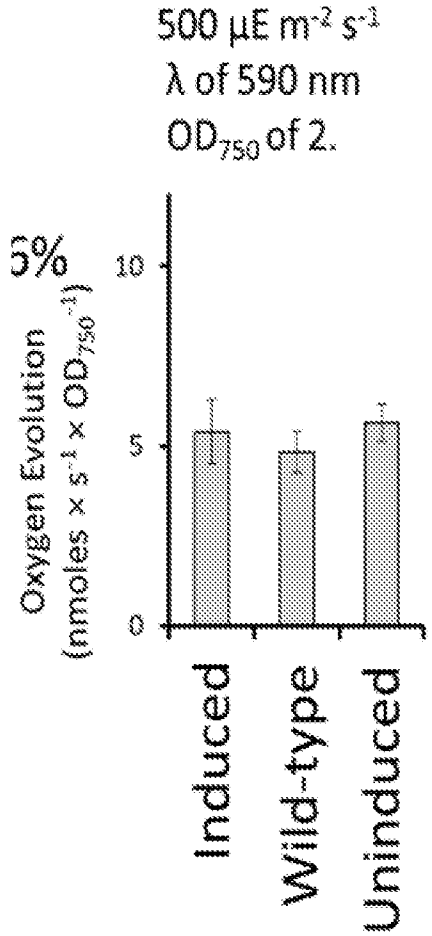
500 µE m-2 s-1
λ of 590 nm
OD750 of 2.
5%
Oxygen Evolution
(nmoles × s-1 × OD750-1)
10
5
0
Induced
Wild-type
Uninduced

FIG. 10

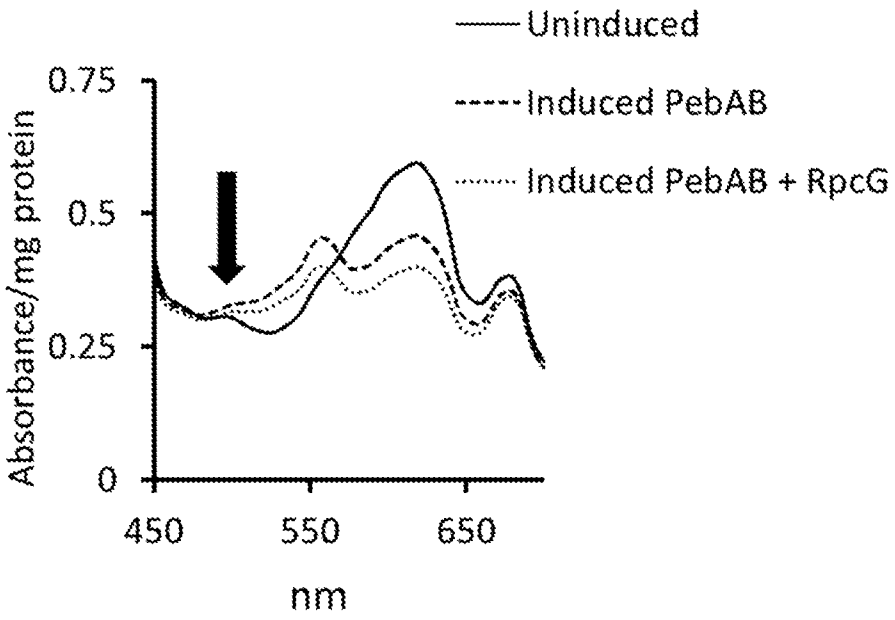
Uninduced
Induced PebAB
Induced PebAB + RpcG
Absorbance/mg protein
0.75
0.5
0.25
0
450
550
650
nm

FIG. 11

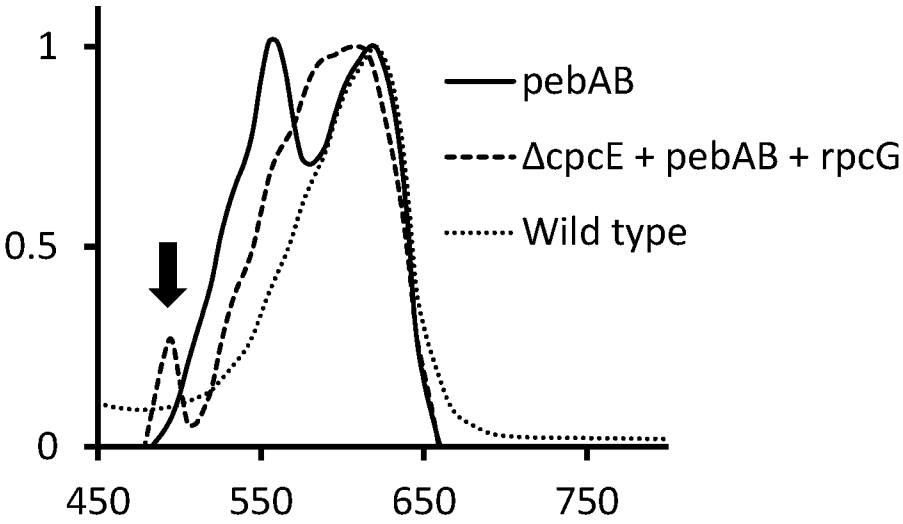
pebAB
ΔcpcE + pebAB + rpcG
Wild type
1
0.5
0
450
550
650
750

FIG. 12A

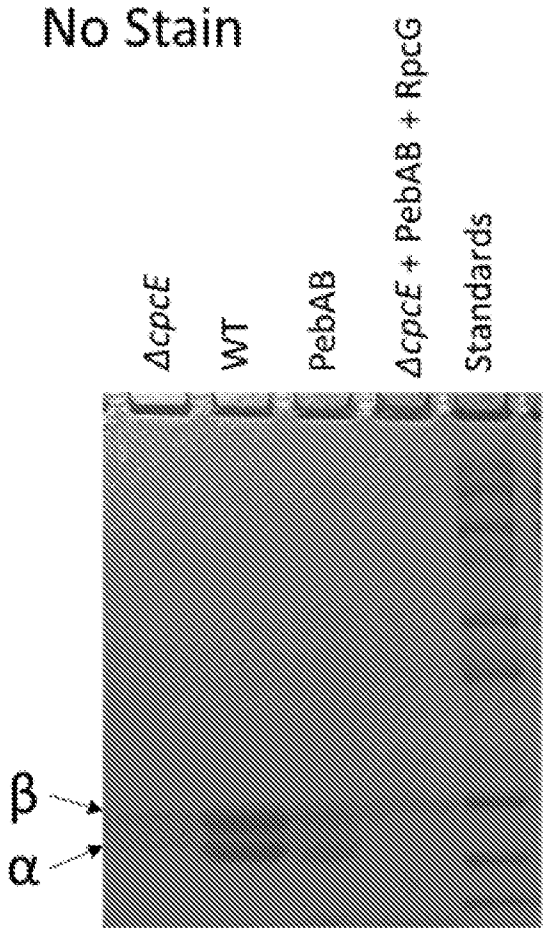

FIG. 12B

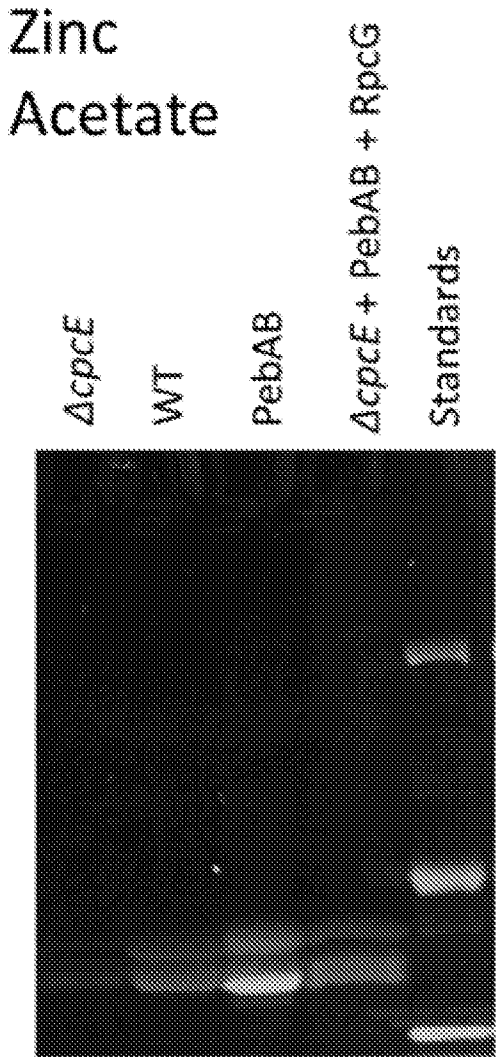

FIG. 12C

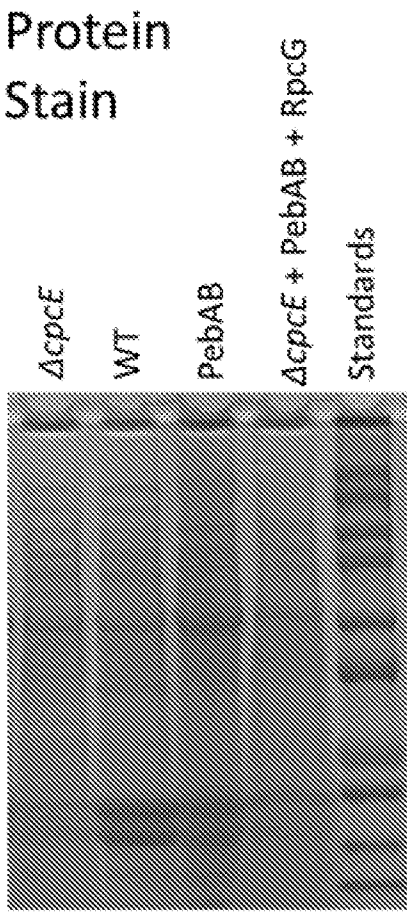

FIG. 13A

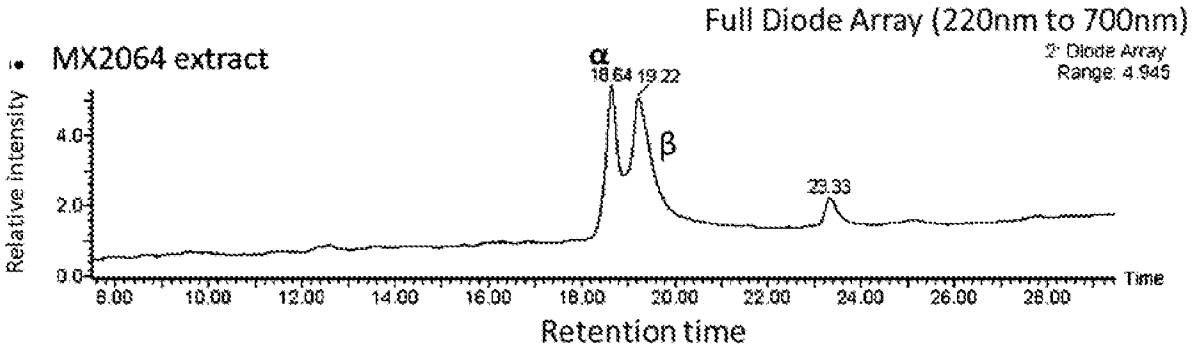

FIG. 13B

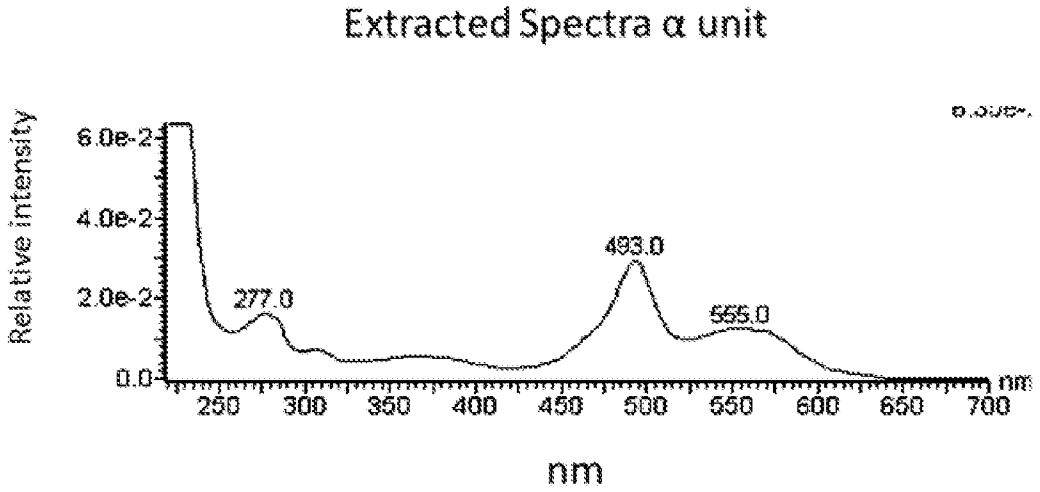

FIG. 13C

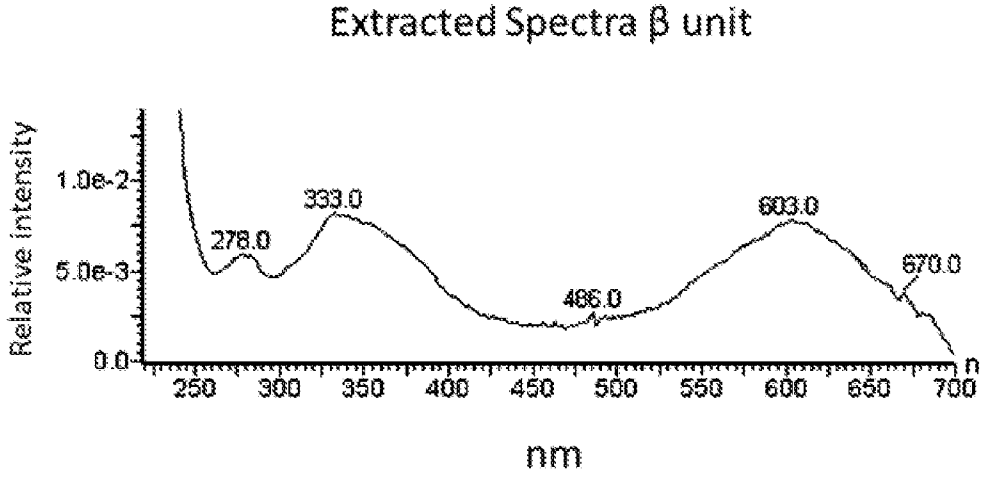
Extracted Spectra β unit
Relative intensity
1.0e-2
5.0e-3
0.0
278.0
333.0
486.0
603.0
670.0
250
300
350
400
450
500
550
600
650
700
nm

FIG. 14

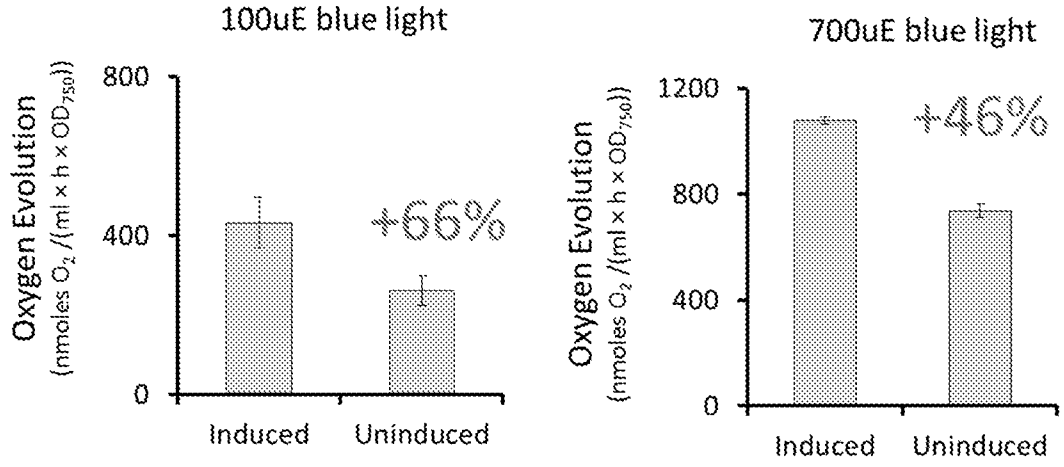
100uE blue light
Oxygen Evolution
(nmoles O_2 /(ml × h × OD_{750}))
800
400
0
+66%
Induced
Uninduced
700uE blue light
Oxygen Evolution
(nmoles O_2 /(ml × h × OD_{750}))
1200
800
400
0
+46%
Induced
Uninduced

FIG. 15

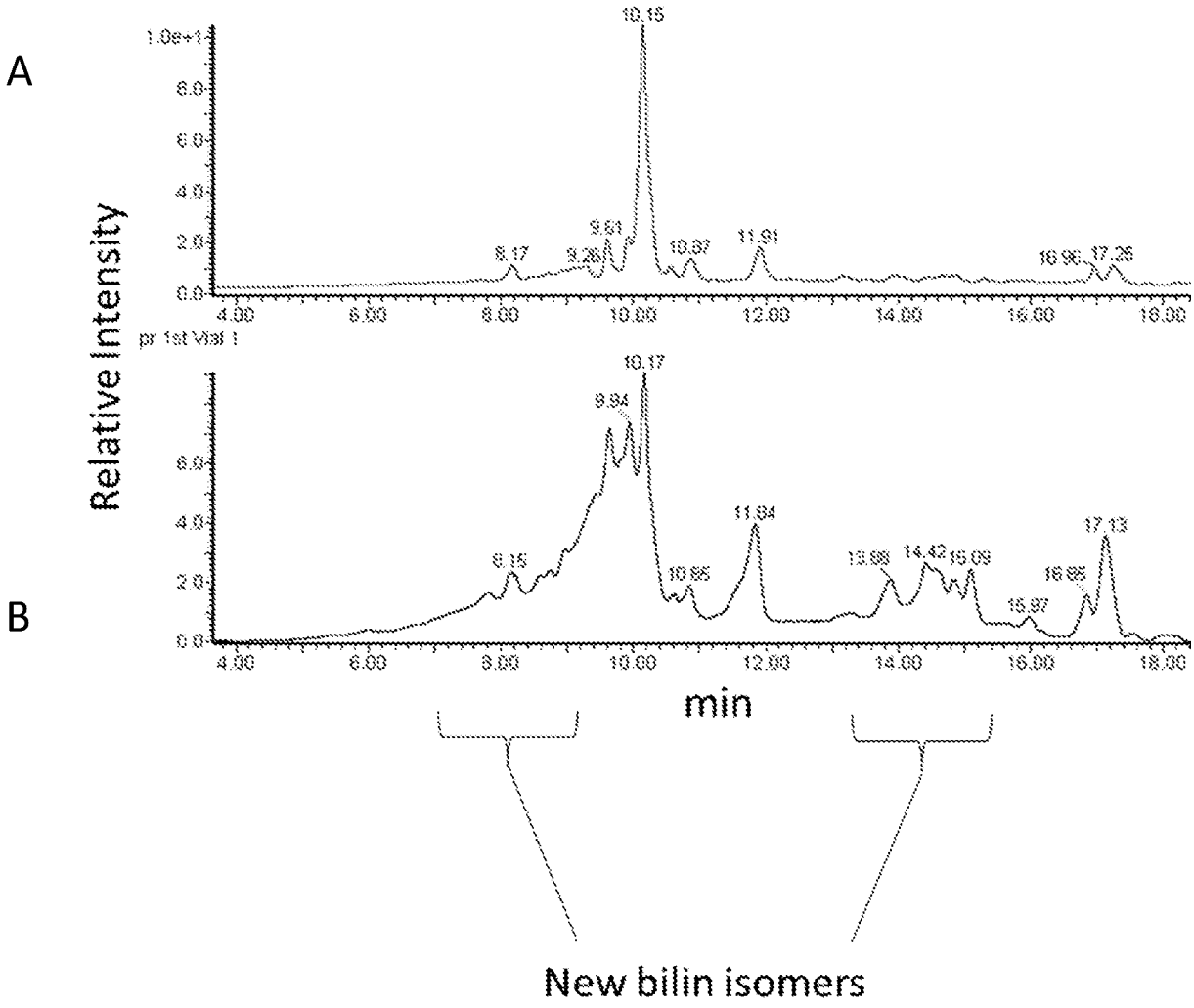
A
B
Relative Intensity
min
10.16
9.61
8.17
9.26
10.07
11.91
16.96
17.26
10.17
9.84
8.15
10.85
11.84
13.68
14.42
15.09
15.97
16.85
17.13
New bilin isomers

FIG. 16A

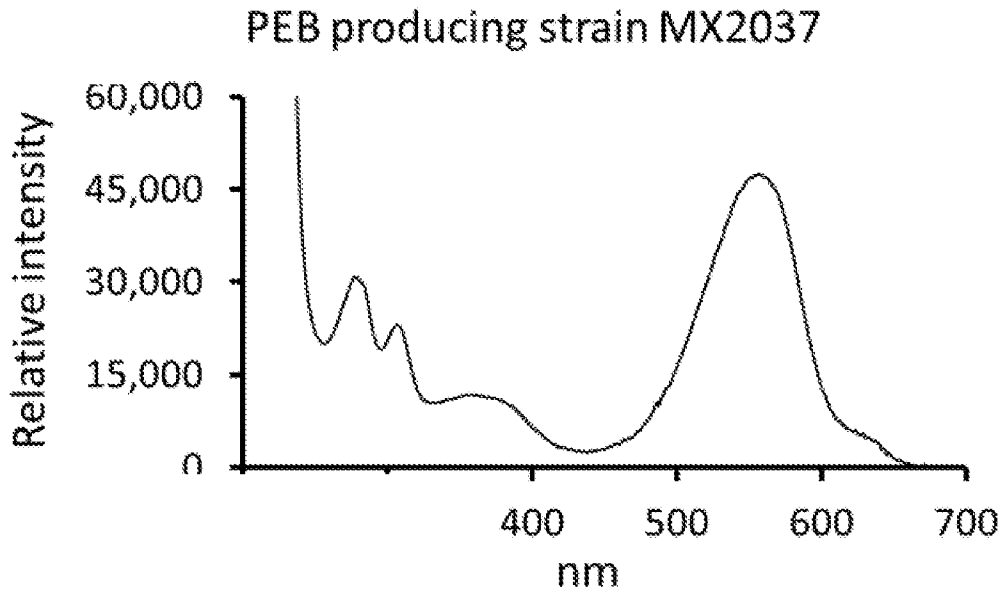

FIG. 16B

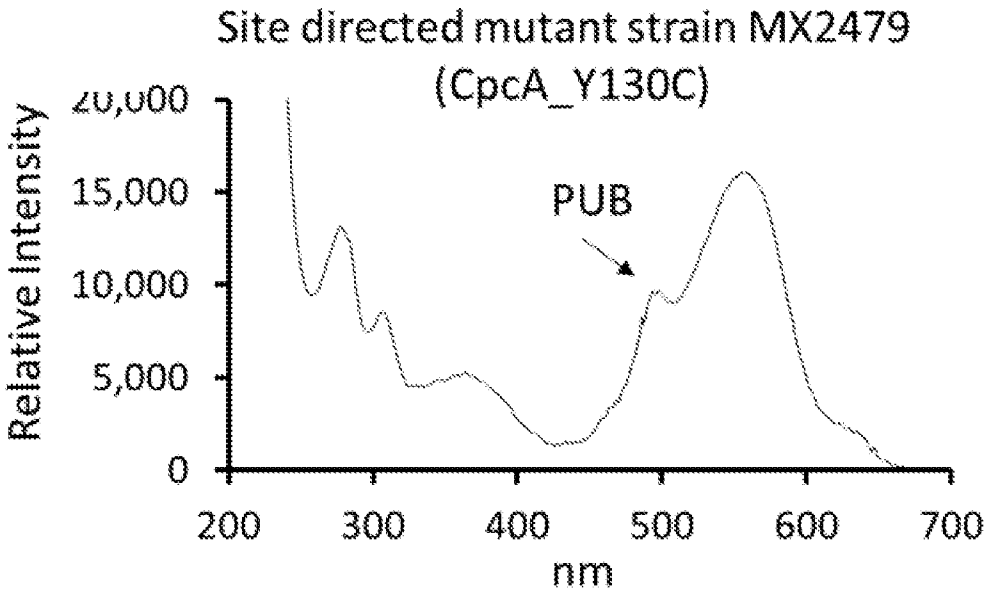

FIG. 17A

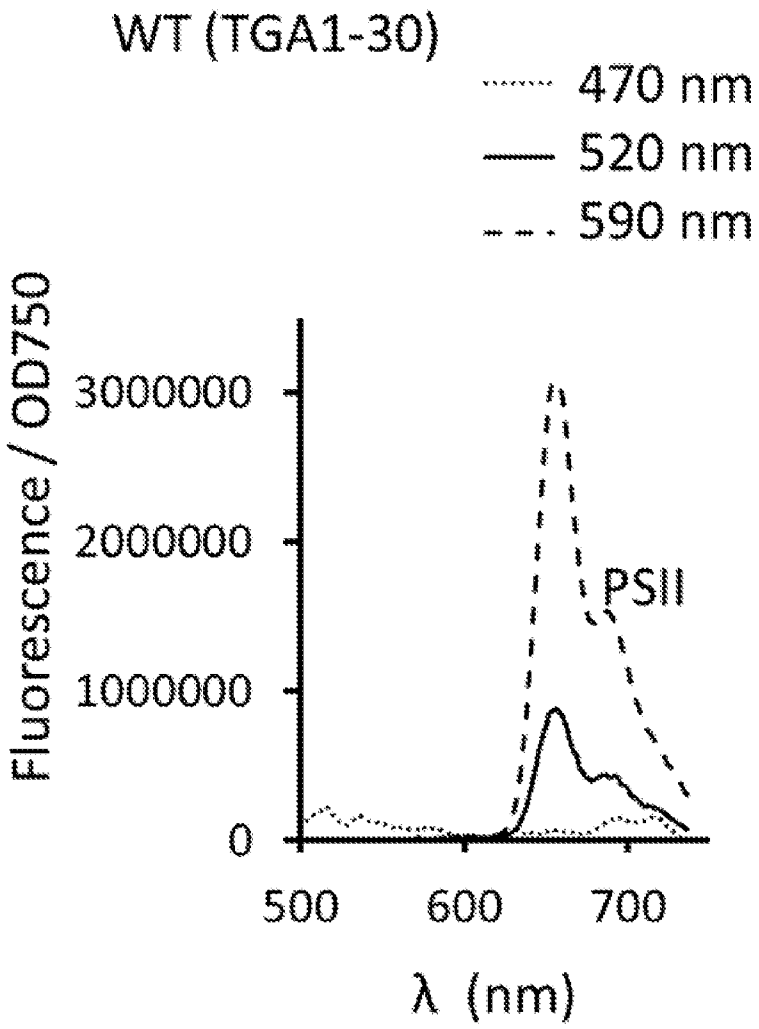
WT (TGA1-30)
470 nm
520 nm
590 nm
Fluorescence / OD750
3000000
2000000
1000000
0
PSII
500
600
700
λ (nm)

FIG. 17B

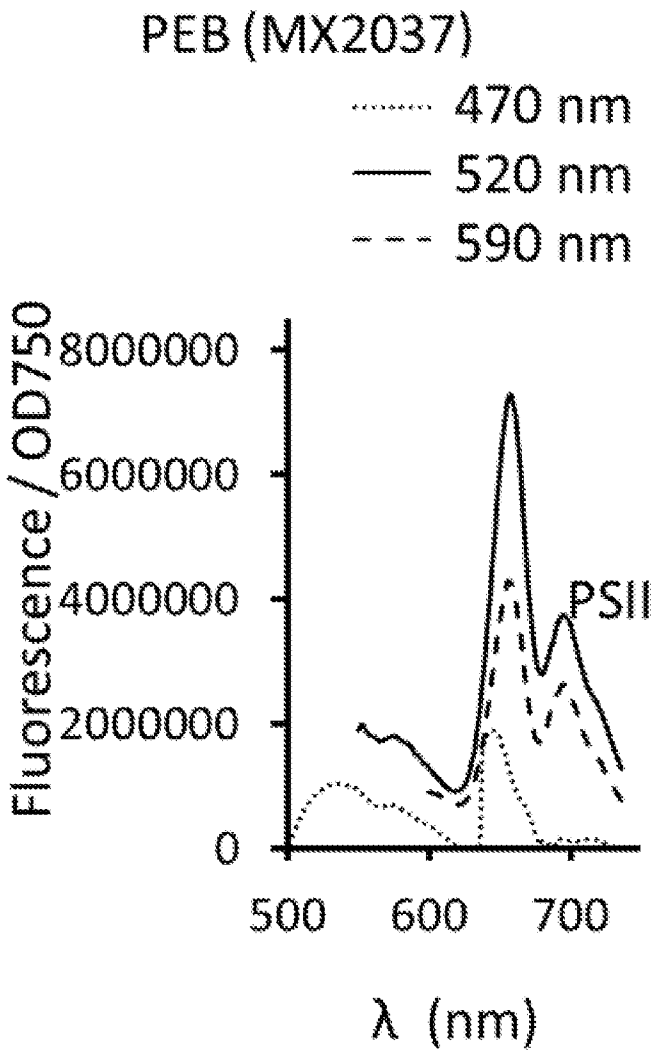
PEB (MX2037)
470 nm
520 nm
590 nm
Fluorescence / OD750
8000000
6000000
4000000
2000000
0
PSII
500
600
700
λ (nm)

FIG. 17C

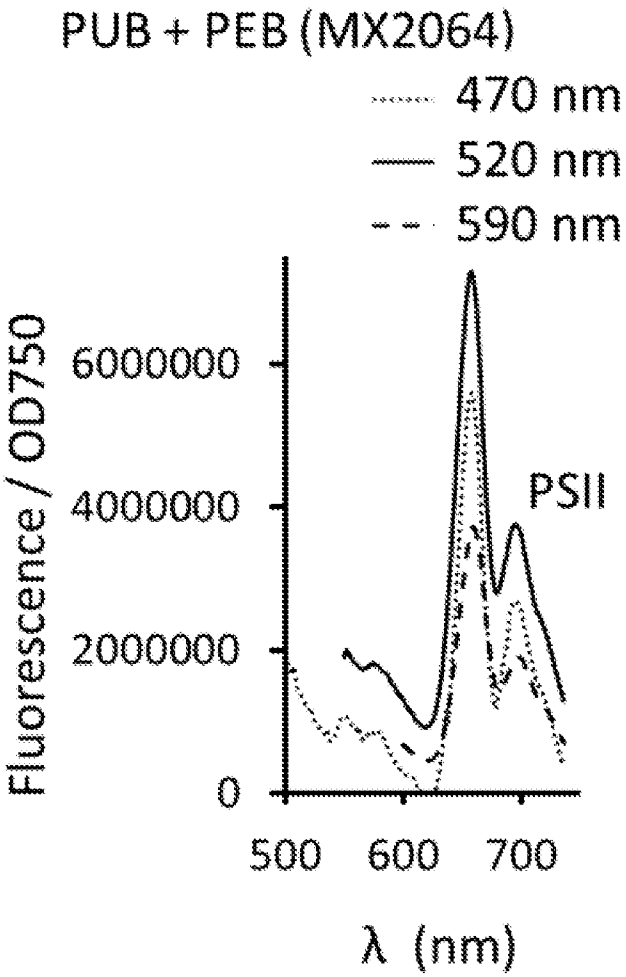
PUB + PEB (MX2064)
470 nm
520 nm
590 nm
Fluorescence / OD750
6000000
4000000
2000000
0
PSII
500
600
700
λ (nm)

FIG. 18A

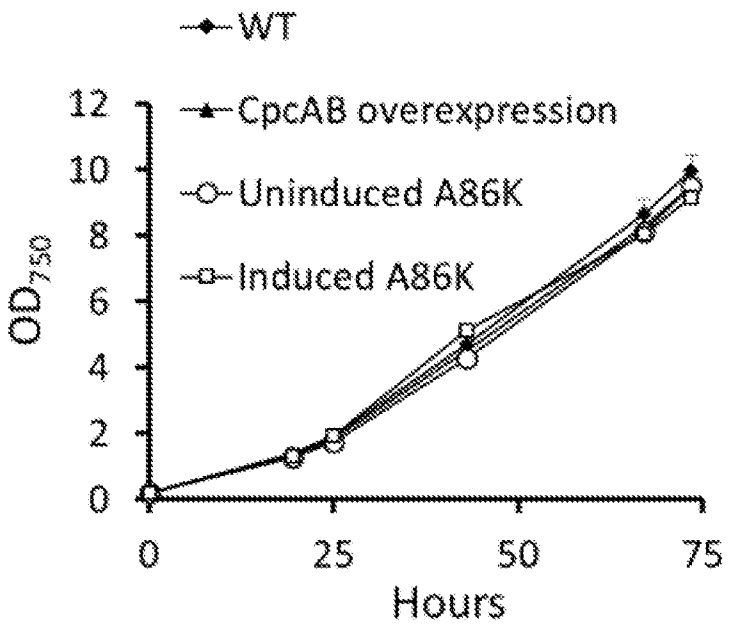

FIG. 18B

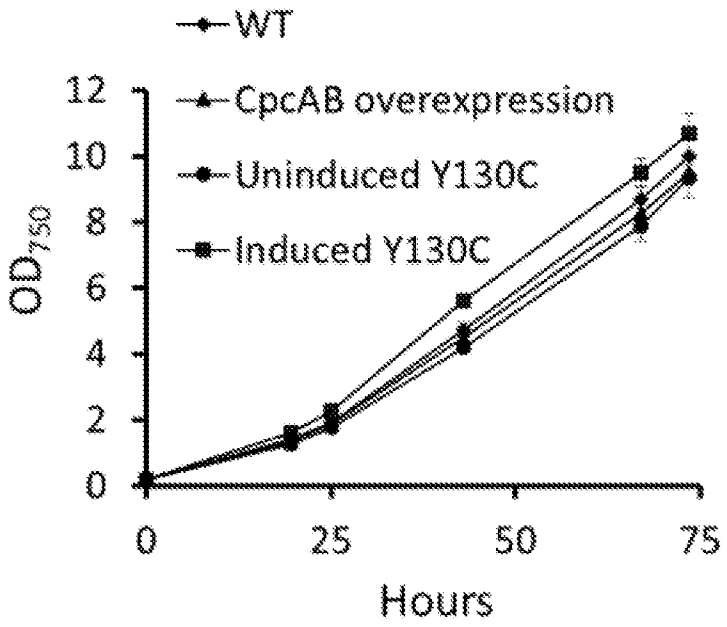

FIG. 18C

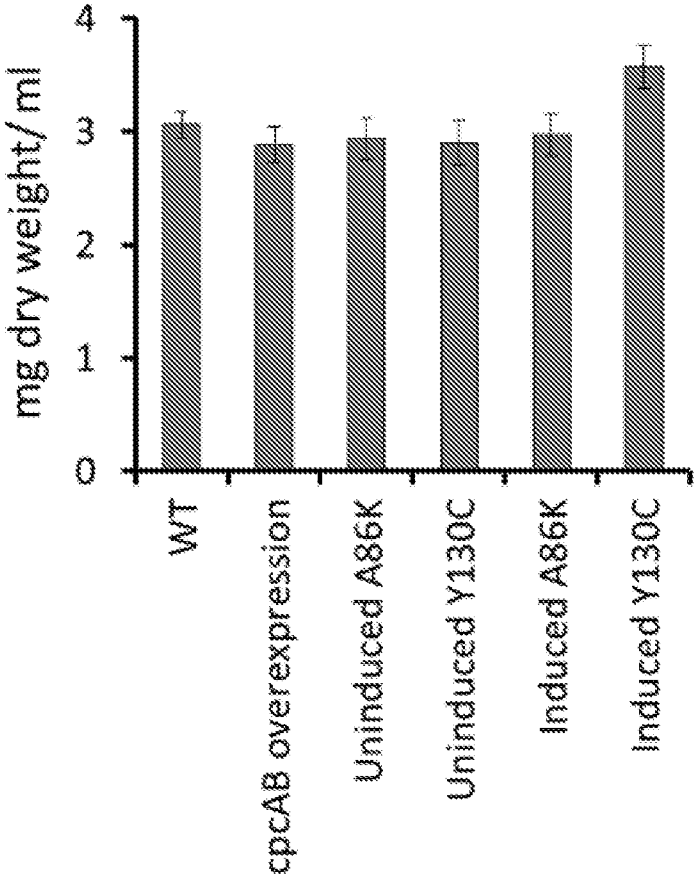
mg dry weight/ml
0
1
2
3
4
WT
cpcAB overexpression
Uninduced A86K
Uninduced Y130C
Induced A86K
Induced Y130C

FIG. 19A

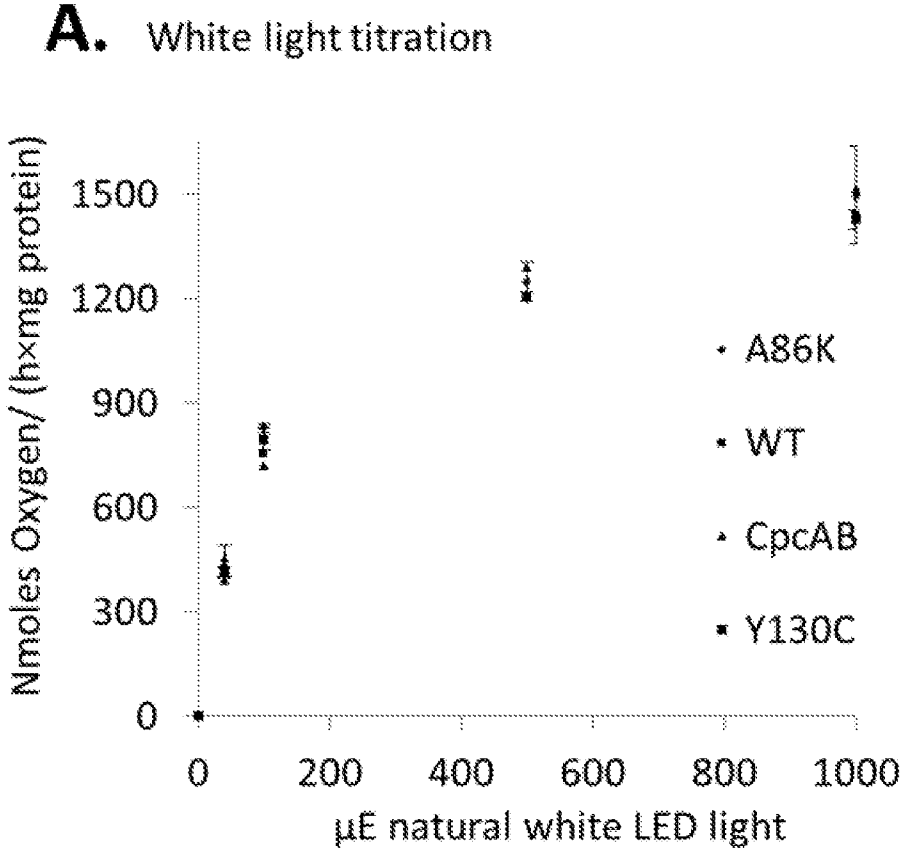
A. White light titration
Nmoles Oxygen/ (h×mg protein)
1500
1200
900
600
300
0
0
200
400
600
800
1000
µE natural white LED light
A86K
WT
CpcAB
Y130C

FIG. 19B

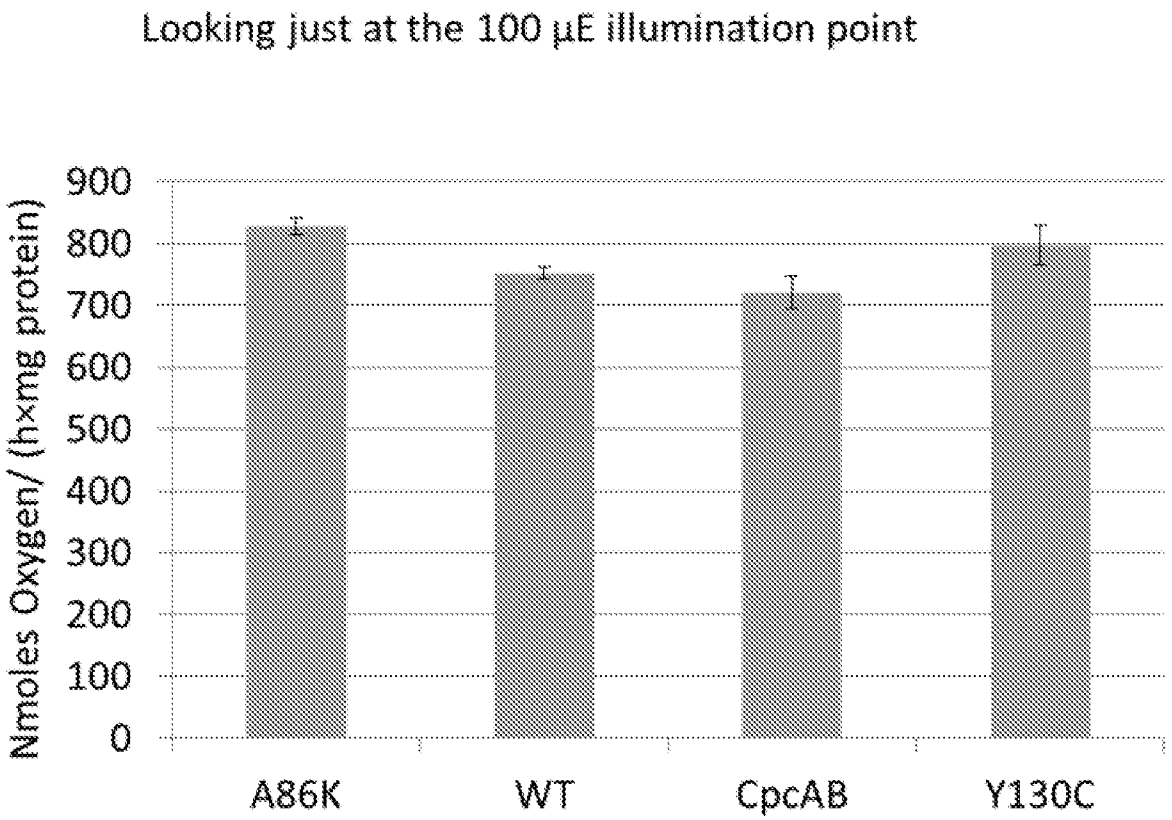
Looking just at the 100 µE illumination point
Nmoles Oxygen/ (h×mg protein)
900
800
700
600
500
400
300
200
100
0
A86K
WT
CpcAB
Y130C

FIG. 20A

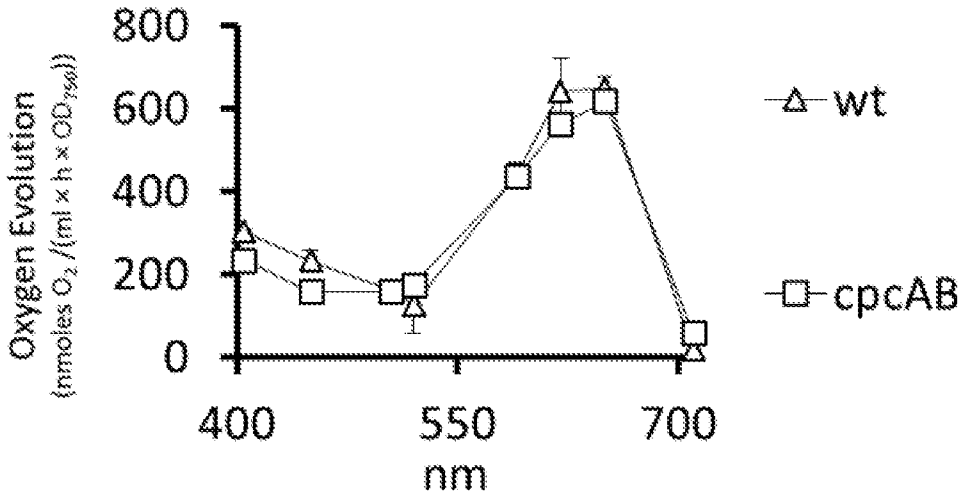

FIG. 20B

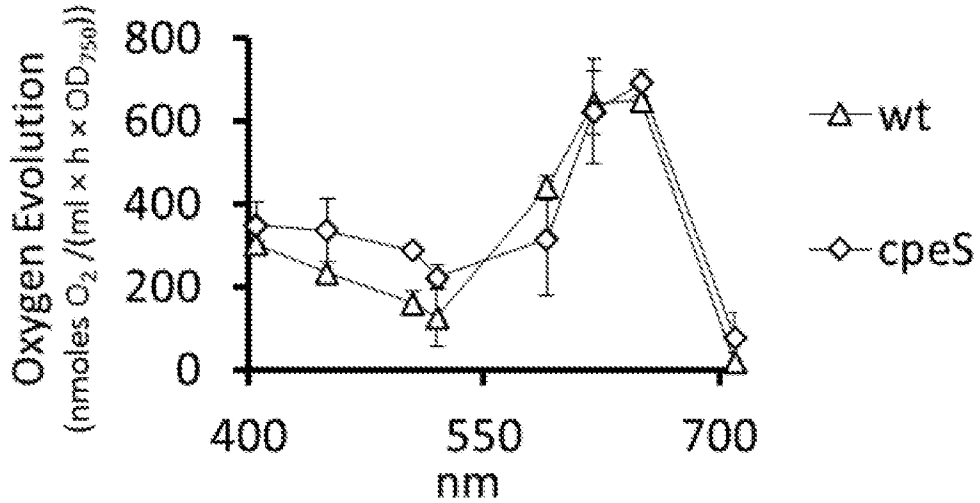

FIG. 20C

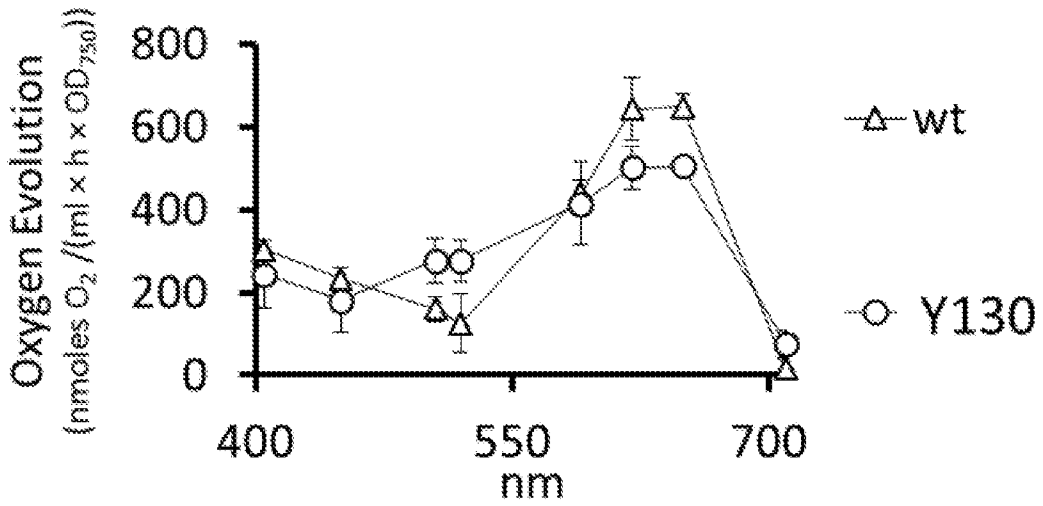
Oxygen Evolution
(nmoles O_2 /(ml × h × OD_{730}))
800
600
400
200
0
400
550
700
nm
wt
Y130

FIG. 21

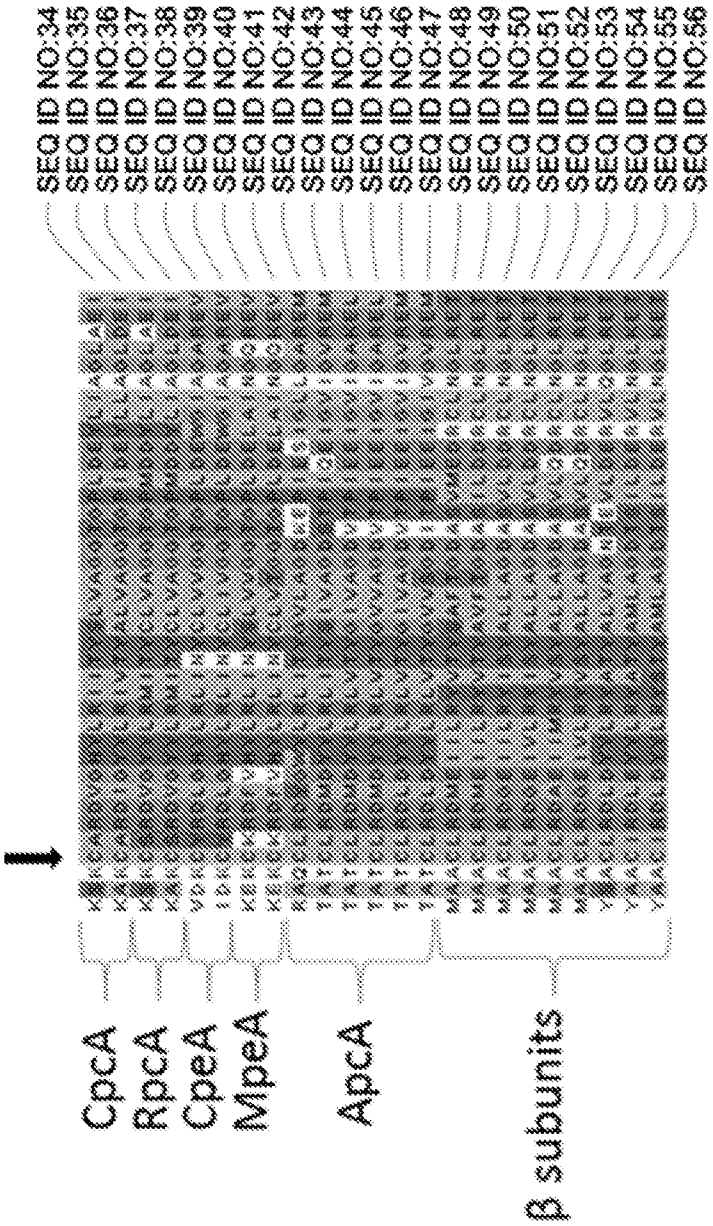
SEQ ID NO:34
SEQ ID NO:35
SEQ ID NO:36
SEQ ID NO:37
SEQ ID NO:38
SEQ ID NO:39
SEQ ID NO:40
SEQ ID NO:41
SEQ ID NO:42
SEQ ID NO:43
SEQ ID NO:44
SEQ ID NO:45
SEQ ID NO:46
SEQ ID NO:47
SEQ ID NO:48
SEQ ID NO:49
SEQ ID NO:50
SEQ ID NO:51
SEQ ID NO:52
SEQ ID NO:53
SEQ ID NO:54
SEQ ID NO:55
SEQ ID NO:56
CpcA
RpcA
CpeA
MpeA
ApcA
β subunits

FIG. 22

SEQ ID NO: 2 – PebA

MFDPFLEELQTGIQARGGISVEVPAGLEHNQSQKGSSTIQSWLWQVPGFRRWRVTRLDA
GDSLQVLNSVAYPDFDLDHPLMGVDLLWFGARQKLVAVLDFQPLVQDKDYLDRHFDGLK
DLNARFPDLNGEETMRSFDPNQYFSSWLLFCRGGSEEADRSLPKAFSAFLKAYWGLHD
EASKEPSSISPGDVERLQNAYDVYSAERDPAHGLFTSHFGKEWSDRFLHEFLFPASQPA

SEQ ID NO: 3 – PebB

MSIDLRASSLDPVQIPGWRWQPFLDEASAALKPFNPSPYPIAETFLQKEGSTGSKAKPVP
VTTATWACSTDKLRQVRCACVEAGMAASVLNFVINPSCRFDLPFFGADLVTLPNGHLLAL
DLQPVDKADPDHTQPVWERLMPLFERWQAELPDGGPIPEEAQPYFSPAFLWTRIPLGEE
GDELIERVIRPAFIDYLQLYLNLVAEAEPVSDDRAELLLSGQKRYTAYRAEKDPARGMLTR
FYGSEWTESYIHGVLFDLEDAA

SEQ ID NO: 1 – RpcG

MFLHVDRKQELHMPIDSVTAALEALDHQDAGVRYHGAWWLGKNRSAEGVPRLVECLLD
ERDKTCTGGYPLRRQAARSLGMIKDSRCLPELLKTLETDDVQLHEATLRALIQIKSDQCSS
SLINYLDRDIPNKPIEALIEALTEQRMWDVSEKIQPFLNDKSERIAGSAAAFFYSYTGEMTY
LNKVISLLDHQNRFIRQSAAFDLARIGTIKATDPILTAKIPNNVKMFAIKAILNKSLSRSNQAD
SIPDTDLASIHSSLFKALDSLARDNFSGNLLIEQDNQIPETYPGDGSTESDLLSNAFDNLRS
PSLTSRKSGIKQLVRGANRFKIDLLDLYFSESDQDITMGLIKAMAELKNPHYANALIDAIGV
EIGNHCQGNIRRVAACALGDINWNAKISSQSLHAVFNKLKWTLHSPEDWGLRYSACLALE
GIGNADSIKLLNEAKAKETDPVLSARLDKAILKSKNKTSIHHIENKKVL

SEQ ID NO: 61 – Variant CpcA (A86K)

MSKTPLTEAVAAADSQGRFLSSTELQVAFGRFRQAASGLAAAKALANNADSLVNGAANA
VYSKFPYTTSTPGNNFASTPEGKAKCKRDIGYYLRIVTYALVAGGTGPIDEYLLAGLDEINK
TFDLAPSWTVEALKYIKANHGLSGDSRDEANSYIDYLINALS*

SEQ ID NO: 62 – Variant CpcA (Y130C)

MSKTPLTEAVAAADSQGRFLSSTELQVAFGRFRQAASGLAAAKALANNADSLVNGAANA
VYSKFPYTTSTPGNNFASTPEGKAKCARDIGYYLRIVTYALVAGGTGPIDEYLLAGLDEINK
TFDLAPSWCVEALKYIKANHGLSGDSRDEANSYIDYLINALS*

SEQ ID NO: 63 – CpcB

MTFDAFTKVVAQADARGEFLSDAQLDALSRLVAEGNKRIDTVNRITGNASSIVANAARALF
AEQPSLIAPGGNAYTNRRMAACLRDMEIILRYVTYAVFTGDASILDDRCLNGLRETYLALG
VPGASVAEGVRKMKDAAVAIVSDRNGITQGDCSAIISELGSYFDKAAAAVA*

FIG. 22 cont.

SEQ ID NO: 64 – CpeS1

MFPLQSYPPMTMVDFFEASRGTWLNRRAVHHLDHQDDEAADSNLVIEPFKNDDPAVRSI
CEALNINMIDSTGGARFWWESNIKKGVRNEDYAAVVIDVPNRDNARKGFLLRDVGYVEK
QAVLSTYVFAEDGVLTITTRYDTNIGIERCWFVTDQIRMRVSSVQCLDGVAMTTYCTEFRC
PTDADINAISEHARQIARSTASIGA*

SEQ ID NO: 65 – CpeS2

MSTILKSMTIEQFVAQSVGKWRSMRSGHSLAFQQFEDVLSEVIIESIEKDDSAVQDLLSTA
TSNQGHSSDIVAPFRMEWSAESDWEPEDPSQVSSGSCLIIPLKKNDYSGILIRSVGYAES
ELAESTYQFLDDGTFLLTTHYEQSMAEERIWFVSDNVRCRSSVLKTSAGSGVLQTSFASE
VRRVKA*

SEQ ID NO: 4 – *pebA* gene with stop codon

ATGTTTGATCCGTTTCTTGAGGAATTACAAACTGGAATTCAAGCCCGCGGTGGCATAT
CAGTTGAAGTTCCGGCCGGGCTGGAACACAATCAATCCCAGAAGGGCTCAAGCACCA
TCCAAAGCTGGCTTTGGCAGGTTCCAGGTTTTCGTCGCTGGCGCGTCACCCGACTTG
ATGCAGGTGACAGCCTTCAAGTTCTGAATTCCGTCGCATATCCCGATTTCGATTTGGA
CCATCCTTTGATGGGTGTTGATCTGCTCTGGTTTGGCGCACGTCAAAAGCTAGTTGC
GGTTCTTGATTTTCAACCACTGGTTCAAGACAAAGACTATCTCGATCGTCATTTTGATG
GTCTGAAAGATCTGAATGCTCGTTTCCCGGATCTAAACGGAGAAGAAACGATGCGAT
CTTTCGATCCGAATCAATACTTCTCATCATGGCTACTTTTTTGCCGTGGAGGTTCTGAA
GAGGCTGACAGGTCACTGCCAAAGGCCTTCAGCGCCTTTTTGAAAGCCTATTGGGGT
TTACACGATGAGGCTTCCAAGGAACCATCCTCAATCTCACCTGGAGATGTGGAACGG
CTTCAGAACGCCTACGACGTGTACAGCGCCGAGCGTGATCCTGCCCATGGATTGTTC
ACCAGCCATTTCGGCAAGGAGTGGTCTGACCGGTTCCTGCACGAATTCCTTTTCCCT
GCCAGTCAGCCCGCATGA

SEQ ID NO: 5 – *pebB* gene with stop codon

ATGAGCATTGATCTCCGCGCGTCGAGCCTTGATCCCGTTCAGATTCCGGGGTGGCGA
TGGCAGCCCTTTCTCGATGAAGCCAGTGCTGCACTCAAGCCGTTCAACCCGTCTCCC
TATCCCATAGCGGAAACGTTTCTGCAAAAGGAGGGCAGCACCGGTTCAAAAGCGAAA
CCCGTTCCGGTGACAACGGCGACCTGGGCCTGTTCCACAGACAAGTTGCGTCAGGT
GCGTTGTGCCTGCGTTGAAGCGGGTATGGCTGCTTCGGTGCTCAATTTTGTGATCAA
CCCGAGCTGTCGGTTCGACCTGCCGTTCTTCGGAGCCGATCTGGTGACGCTTCCAAA
CGGCCATTTGCTCGCTCTGGATCTTCAACCGGTTGACAAGGCTGATCCCGATCACAC
CCAACCCGTGTGGGAGCGACTGATGCCGTTGTTCGAGCGCTGGCAAGCCGAACTCC
CCGATGGAGGCCCCATCCCAGAAGAAGCCCAACCCTATTTCTCACCGGCGTTTCTCT
GGACCCGCATCCCGCTTGGGGAGGAGGGGGATGAACTGATTGAACGCGTGATCCGC
CCGGCCTTCATCGACTATCTGCAGCTTTACCTCAACCTCGTGGCTGAAGCGGAACCC
GTGTCTGACGACCGTGCGGAATTGCTCCTTTCAGGCCAAAAGCGCTACACCGCGTAT
CGCGCCGAGAAGGATCCAGCCCGCGGCATGTTGACGCGGTTCTACGGGAGCGAGT
GGACAGAGTCGTACATCCATGGCGTGCTGTTCGATCTCGAGGACGCCGCTTAA

FIG. 22 cont.

SEQ ID NO: 7 – *RpcG* with stop codon

ATGTTTCTCCATGTCGACAGGAAACAGGAATTAcaTATGCCAATTGACTCAGTTACAGC
CGCTCTTGAAGCCCTCGACCACCAAGATGCGGGTGTTCGGTATCACGGTGCTTGGTG
GCTCGGGAAAAACAGGTCGGCTGAGGGAGTACCACGATTGGTGGAATGCCTTCTAG
ACGAAAGGGACAAAACATGTACAGGCGGATACCCCCTAAGAAGGCAAGCAGCAAGAT
CACTGGGAATGATCAAAGACTCACGCTGTTTACCAGAGCTTCTTAAAACACTAGAAAC
AGATGACGTGCAATTGCATGAAGCAACACTTAGAGCCCTAATTCAAATCAAGAGTGAT
CAATGCTCAAGCTCACTCATTAACTACCTTGACCGAGATATTCCCAACAAACCAATAG
AAGCGCTTATAGAAGCCTTAACAGAGCAAAGAATGTGGGATGTTTCAGAAAAGATCCA
ACCCTTTCTTAACGACAAATCAGAAAGGATCGCTGGCTCTGCAGCAGCTTTTTTCTAC
AGCTACACCGGTGAGATGACCTATTTAAACAAAGTTATCTCACTTCTTGATCACCAGA
ATCGCTTCATCAGGCAATCCGCTGCATTCGACCTAGCCCGTATCGGAACAATCAAAG
CAACAGATCCAATCCTGACTGCCAAGATCCCCAACAACGTCAAGATGTTTGCCATAAA
AGCCATACTCAATAAATCGCTCAGCCGAAGCAATCAAGCAGATTCTATTCCAGATACC
GACCTCGCATCAATTCATTCCTCCCTCTTCAAAGCACTTGACAGTCTCGCCAGAGACA
ACTTTTCGGGGAACCTATTGATTGAGCAAGACAACCAAATTCCAGAAACCTATCCAGG
AGACGGCTCAACAGAGAGCGATCTACTTTCAAATGCATTCGACAACCTAAGGTCACCA
TCCTTGACGAGCAGAAAATCAGGCATAAAACAACTCGTCCGTGGTGCTAATCGTTTCA
AAATCGATCTTCTTGATCTGTACTTCTCAGAATCAGATCAAGACATCACAATGGGGCT
GATCAAGGCCATGGCTGAACTCAAAAATCCCCATTACGCAAACGCACTTATTGATGCT
ATTGGGGTCGAAATTGGCAATCATTGCCAAGGAAACATTCGACGCGTCGCAGCGTGC
GCCCTTGGTGACATCAATTGGAACGCGAAGATTTCGTCGCAATCACTGCATGCCGTTT
TCAACAAACTCAAATGGACACTTCATTCACCTGAAGACTGGGGTTTGCGCTACAGCGC
ATGCTTGGCGCTGGAAGGAATTGGCAATGCCGATTCGATTAAACTCTTAAATGAAGCT
AAAGCAAAAGAAA
CAGATCCAGTCCTCTCTGCACGCCTTGACAAGGCAATACTAAAATCAAAAAATAAGAC
TTCTATCCATCACATCGAAAACAAAAAAGTTCTCTAA

SEQ ID NO: 66 – Variant *CpcA* (A86K) gene with stop codon

ATGTCCAAGACTCCTCTGACCGAAGCTGTCGCTGCTGCTGATTCGCAAGGGCGTTTT
CTGAGCAGCACTGAACTGCAAGTTGCATTTGGTCGTTTCCGTCAAGCTGCTTCTGGTT
TGGCAGCGGCTAAGGCGTTGGCAAACAATGCTGACAGCTTGGTCAACGGTGCAGCG
AACGCTGTTTACAGCAAGTTCCCCTACACCACCAGCACGCCTGGCAACAACTTTGCAT
CGACGCCGGAAGGCAAAGCGAAGTGTAAGCGTGACATTGGTTACTATCTGCGGATTG
TGACCTATGCATTGGTTGCGGGTGGCACGGGTCCGATTGATGAGTACCTGTTGGCAG
GTCTTGATGAGATCAACAAGACCTTCGACTTGGCGCCGAGCTGGTGTGTGGAAGCGC
TGAAGTACATCAAAGCGAATCATGGCTTGAGTGGTGACTCTCGCGATGAAGCCAACT
CCTACATCGACTACCTCATCAATGCCCTCAGCTAG

FIG. 22 cont.

SEQ ID NO: 67 – Variant *CpcA* (Y130C) gene with stop codon

ATGTCCAAGACTCCTCTGACCGAAGCTGTCGCTGCTGCTGATTCGCAAGGGCGTTTT
CTGAGCAGCACTGAACTGCAAGTTGCATTTGGTCGTTTCCGTCAAGCTGCTTCTGGTT
TGGCAGCGGCTAAGGCGTTGGCAAACAATGCTGACAGCTTGGTCAACGGTGCAGCG
AACGCTGTTTACAGCAAGTTCCCCTACACCACCAGCACGCCTGGCAACAACTTTGCAT
CGACGCCGGAAGGCAAAGCGAAGTGTGCGCGTGACATTGGTTACTATCTGCGGATT
GTGACCTATGCATTGGTTGCGGGTGGCACGGGTCCGATTGATGAGTACCTGTTGGCA
GGTCTTGATGAGATCAACAAGACCTTCGACTTGGCGCCGAGCTGGTGTGTGGAAGCG
CTGAAGTACATCAAAGCGAATCATGGCTTGAGTGGTGACTCTCGCGATGAAGCCAAC
TCCTACATCGACTACCTCATCAATGCCCTCAGCTAG

SEQ ID NO: 68 – *CpcB* gene with stop codon

ATGACTTTTGATGCTTTCACCAAGGTGGTGGCACAAGCCGATGCCCGTGGCGAATTT
TTGAGCGACGCCCAACTGGACGCGCTGAGCCGCTTGGTTGCAGAAGGCAACAAACG
GATTGATACGGTCAACCGCATCACCGGTAATGCTTCGTCGATCGTCGCTAACGCAGC
GCGTGCATTGTTTGCAGAGCAACCTTCTCTGATTGCTCCTGGCGGCAACGCATACAC
GAACCGTCGGATGGCGGCTTGTCTGCGCGACATGGAAATCATTCTCCGCTACGTGAC
CTACGCGGTCTTCACCGGCGATGCTTCCATTCTCGACGACCGCTGTTTGAACGGTCT
GCGTGAGACCTACTTGGCTCTGGGCGTGCCCGGTGCATCGGTGGCAGAAGGCGTTC
GCAAGATGAAAGACGCAGCTGTGGCGATTGTGAGCGACCGCAACGGCATCACCCAA
GGTGACTGTTCAGCGATCATTTCCGAGCTGGGCAGCTACTTCGACAAAGCTGCTGCT
GCAGTTGCCTAG

SEQ ID NO: 69 – *CpeS1* gene with stop codon

ATGTTTCCCCTCCAGTCATATCCACCAATGACGATGGTGGATTTTTTCGAAGCAAGCC
GTGGGACCTGGTTGAACCGACGTGCTGTTCATCATTTGGATCACCAGGATGATGAAG
CAGCAGATTCTAATCTTGTTATCGAACCATTTAAAAATGATGATCCGGCAGTTCGCAG
CATTTGCGAAGCCCTAAACATCAACATGATCGACAGTACTGGTGGAGCTAGATTTTGG
TGGGAAAGTAATATTAAAAAAGGAGTCCGCAACGAAGATTATGCTGCTGTTGTCATCG
ATGTACCCAACCGAGATAATGCTCGAAAGGGTTTCTTACTACGAGATGTAGGATATGT
TGAAAAGCAGGCGGTATTGAGCACTTACGTTTTTGCCGAAGATGGCGTGTTGACGAT
CACTACAAGATATGACACGAATATTGGAATTGAACGATGCTGGTTTGTGACTGATCAG
ATCCGAATGCGTGTCAGTTCTGTCCAATGCTTGGATGGTGTCGCAATGACTACCTACT
GCACTGAATTTCGCTGTCCAACAGATGCTGATATCAATGCCATATCTGAGCATGCCAG
GCAGATCGCTCGTTCGACTGCATCTATTGGAGCTTAA

FIG. 22 cont.

SEQ ID NO: 70 – *CpeS2* gene with stop codon

ATGAGCACAATATTAAAAAGTATGACGATTGAGCAATTTGTTGCTCAAAGTGTGGGTA
AATGGCGCTCCATGAGATCAGGCCATTCTCTCGCTTTTCAACAATTTGAAGACGTTCT
TAGCGAAGTAATTATTGAATCCATCGAGAAAGACGATTCTGCTGTTCAAGATTTACTCT
CAACCGCAACTTCTAACCAAGGACATAGCTCCGACATCGTCGCGCCATTCAGGATGG
AATGGTCAGCTGAAAGTGACTGGGAGCCCGAAGATCCATCTCAAGTTTCATCAGGCT
CGTGCCTGATCATCCCACTGAAAAAAAATGATTATTCTGGCATCTTGATCAGAAGTGT
GGGGTATGCTGAATCCGAATTAGCAGAGTCGACATACCAGTTTTTAGACGATGGCAC
ATTCTTGCTTACAACGCATTATGAGCAATCAATGGCAGAGGAAAGAATCTGGTTTGTT
TCAGACAATGTTCGGTGCAGATCATCTGTATTGAAGACATCTGCAGGCTCAGGAGTTC
TACAAACTTCATTTGCCTCTGAAGTCAGACGAGTCAAGGCCTAG

FIG. 22 cont.

SEQ ID NO: 6 – a pathway for PEB biosynthesis; includes or encodes *lacI*, *pebA*, *pebB*, and the streptomycin resistance cassette (inserted into neutral site 1 of *Syn* 7942 strain MX2037)

CATCGCTTGCAATTCGCGCTAACTTACATTAATTGCGTTGCGCTCATTGACCACTCTC
CAAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTATCGGCCAGAGCACGCGGGG
AGGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCGTTTCACCAGCGACACGGGCA
GCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAGTTGCAACAGTCGATCCACGG
ACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGTGGTCAGAGGCGGGATATAAC
AACTCGAATCTTCAGTATCATCATAGCCCACGACACTAATATCTGCGCCGACGCGGA
GGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGCCATCTGGTCATTCGCGACC
AGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTGCATTGTTTGCTGGAAACCC
GACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGGTTGGATCTGATTGCGGGT
GAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGCACTCACGCTGGAGAGCGGGC
CTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGACCAGATGCTCCACACCCAAG
CGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAATGGGGGTTTGATCGGAGACG
TCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCTTCAACAGCGATAGCATCTTG
GTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACACGCTGAGCCAGGAGGTTGT
GGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCAACCATGGAGACGACCACGC
TTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCAACAATTTGACTCGGGGCGT
GCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAAGCTCTGTTTTCCGGCCAGC
TGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGCCATCGCAGCCTCGACTTTT
TCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAACCACGCGACTGACAGTTTGG
TAGCTCACACCTGCGTATTCTGCAACATCATACAGCGTGACTGGCTTCACATTGACCA
TCCTGAATTGACTCTCTTCCGGG
CGCTATCATGCCATACCGCGAAAGGTTTTGCACCATTCGATGGTGTCAACGTACGACT
GCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATG
GCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTC
TGGATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGAGC
TGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATT
GTCGACAGGAAACAGGAATTACATATGTTTGATCCGTTTCTTGAGGAATTACAAACTG
GAATTCAAGCCCGCGGTGGCATATCAGTTGAAGTTCCGGCCGGGCTGGAACACAATC
AATCCCAGAAGGGCTCAAGCACCATCCAAAGCTGGCTTTGGCAGGTTCCAGGTTTTC
GTCGCTGGCGCGTCACCCGACTTGATGCAGGTGACAGCCTTCAAGTTCTGAATTCCG
TCGCATATCCCGATTTCGATTTGGACCATCCTTTGATGGGTGTTGATCTGCTCTGGTT
TGGCGCACGTCAAAAGCTAGTTGCGGTTCTTGATTTTCAACCACTGGTTCAAGACAAA
GACTATCTCGATCGTCATTTTGATGGTCTGAAAGATCTGAATGCTCGTTTCCCGGATC
TAAACGGAGAAGAAACGATGCGATCTTTCGATCCGAATCAATACTTCTCATCATGGCT
ACTTTTTTGCCGTGGAGGTTCTGAAGAGGCTGACAGGTCACTGCCAAAGGCCTTCAG
CGCCTTTTTGAAAGCCTATTGGGGTTTACACGATGAGGCTTCCAAGGAACCATCCTCA
ATCTCACCTGGAGATGTGGAACGGCTTCAGAACGCCTACGACGTGTACAGCGCCGA
GCGTGATCCTGCCCATGGATTGTTCACCAGCCATTTCGGCAAGGAGTGGTCTGACCG
GTTCCTGCACGAATTCCTTTTCCCTGCCAGTCAGCCCGCATGAGCATTGATCTCCGC
GCGTCGAGCCTTGATCCCGTTCAGATTCCGGGGTGGCGATGGCAGCCCTTTCTCGAT
GAAGCCAGTGCTGCACTCAAGCCGTTCAACCCGTCTCCCTATCCCATAGCGGAAACG
TTTCTGCAAAAGGAGGGCAGCACCGGTTCAAAAGCGAAACCCGTTCCGGTGACAACG

FIG. 22 cont.

GCGACCTGGGCCTGTTCCACAGACAAGTTGCGTCAGGTGCGTTGTGCCTGCGTTGAA
GCGGGTATGGCTGCTTCGGTGCTCAATTTTGTGATCAACCCGAGCTGTCGGTTCGAC
CTGCCGTTCTTCGGAGCCGATCTGGTGACGCTTCCAAACGGCCATTTGCTCGCTCTG
GATCTTCAACCGGTTGACAAGGCTGATCCCGATCACACCCAACCCGTGTGGGAGCGA
CTGATGCCGTTGTTCGAGCGCTGGCAAGCCGAACTCCCCGATGGAGGCCCCATCCC
AGAAGAAGCCCAACCCTATTTCTCACCGGCGTTTCTCTGGACCCGCATCCCGCTTGG
GGAGGAGGGGGATGAACTGATTGAACGCGTGATCCGCCCGGCCTTCATCGACTATC
TGCAGCTTTACCTCAACCTCGTGGCTGAAGCGGAACCCGTGTCTGACGACCGTGCG
GAATTGCTCCTTTCAGGCCAAAAGCGCTACACCGCGTATCGCGCCGAGAAGGATCCA
GCCCGCGGCATGTTGACGCGGTTCTACGGGAGCGAGTGGACAGAGTCGTACATCCA
TGGCGTGCTGTTCGATCTCGAGGACGCCGCTTAAACTAGTCATCGAGCTAGCAAGCT
TGGCCGGATCCGGCCGGATCCGGAGTTTGTAGAAACGCAAAAAGGCCATCCGTCAG
GATGGCCTTCTGCTTAATTTGATGCCTGGCAGTTTATGGCGGGCGTCCTGCCCGCCA
CCCTCCGGGCCGTTGCTTCGCAACGTTCAAATCCGCTCCCGGCGGATTTGTCCTACT
CAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTG
AGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCTACTCTCGGTACCCGTCGGCTTGA
ACGAATTGTTAGACATTATTTGCCGACTACCTTGGTGATCTCGCCTTTCACGTAGTGG
ACAAATTCTTCCAACTGATCTGCGCGCGAGGCCAAGCGATCTTCTTCTTGTCCAAGAT
AAGCCTGTCTAGCTTCAAGTATGACGGGCTGATACTGGGCCGGCAGGCGCTCCATTG
CCCAGTCGGCAGCGACATCCTTCGGCGCGATTTTGCCGGTTACTGCGCTGTACCAAA
TGCGGGACAACGTAAGCACTACATTTCGCTCATCGCCAGCCCAGTCGGGCGGCGAG
TTCCATAGCGTTAAGGTTTCATTTAGCGCCTCAAATAGATCCTGTTC
AGGAACCGGATCAAAGAGTTCCTCCGCCGCTGGACCTACCAAGGCAACGCTATGTTC
TCTTGCTTTTGTCAGCAAGATAGCCAGATCAATGTCGATCGTGGCTGGCTCGAA
GATACCTGCAAGAATGTCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGCTTAGCT
GGATAACGCCACGGAATGATGTCGTCGTGCACAACAATGGTGACTTCTACAGCGCGG
AGAATCTCGCTCTCTCCAGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAAAGCT
CGCCGCGTTGTTTCATCAAGCCTTACGGTCACCGTAACCAGCAAATCAATATCACTGT
GTGGCTTCAGGCCGCCATCCACTGCGGAGCCGTACAAATGTACGGCCAGCAACGTC
GGTTCGAGATGGCGCTCGATGACGCCAACTACCTCTGATAGTTGAGTCGATACTTCG
GCGATCACCGCTTCCCTCATGATGTTTAACTTTGTTTTAGGGCGACTGCCCTGCTGCG
TAACATCGTTGCTGCTCCATAACATCAAACATCGACCCACGGCGTAACGCGCTTGCT
GCTTGGATGCCCGAGGCATAGACTGTACCCCAAAAAAACAGTCATAACAAGCCATGA
AAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGC
GTGAGCGCATACGCTACTTGCATTACAGCTTACGAACCGAACAGGCTTATGTCCACT
GGGTTCGTGCCTTCATCCGTTTCCACGGTGTGCGTCACCCGGCAACCTTGGGCAGCA
GCGAAGTCGAGGCATTTCTGTCCTGGCTGGCGAACGAGCGCAAGGTTTCGAATTCAC
ATACGCGGCCGCCTGGGCCTTGAGCTCGAATT

FIG. 22 cont.

SEQ ID NO: 8 - includes or encodes *lacI, rpcG*, and the gentamycin resistance cassette (inserted into neutral site 4 of *Syn* 7942 strain MX2064)

TTGACCACTCTCCAAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTATCGGCCAG
AGCACGCGGGGAGGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCGTTTCACCA
GCGACACGGGCAGCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAGTTGCAACA
GTCGATCCACGGACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGTGGTCAGAG
GCGGGATATAACAACTCGAATCTTCAGTATCATCATAGCCCACGACACTAATATCTGC
GCCGACGCGGAGGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGCCATCTGGT
CATTCGCGACCAGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTGCATTGTTTG
CTGGAAACCCGACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGGTTGGATCTG
ATTGCGGGTGAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGCACTCACGCTGG
AGAGCGGGCCTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGACCAGATGCTCC
ACACCCAAGCGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAATGGGGGTTTGA
TCGGAGACGTCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCTTCAACAGCGAT
AGCATCTTGGTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACACGCTGAGCCAG
GAGGTTGTGGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCAACCATGGAGAC
GACCACGCTTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCAACAATTTGACT
CGGGGCGTGCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAAGCTCTGTTTTC
CGGCCAGCTGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGCCATCGCAGCC
TCGACTTTTTCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAACCACGCGACTG
ACAGTTTGGTAGCTCACACCTGCGTATTCTGCAACATCATACAGCGTGACTGGCTTCA
CATTGACCATCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGT
TTTGCACCATTCGATGGTGTCAACGTACGACTGCACGGTGCACCAATGCTTCTGGCG
TCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGCATAAT
TCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCATAA
CGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAA
TGTGTGGAATTGTGAGCGGATAACAATTGTCGACAGGAAACAGGAATTACATATGCCA
ATTGACTCAGTTACAGCCGCTCTTGAAGCCCTCGACCACCAAGATGCGGGTGTTCGG
TATCACGGTGCTTGGTGGCTCGGGAAAAACAGGTCGGCTGAGGGAGTACCACGATT
GGTGGAATGCCTTCTAGACGAAAGGGACAAAACATGTACAGGCGGATACCCCCTAAG
AAGGCAAGCAGCAAGATCACTGGGAATGATCAAAGACTCACGCTGTTTACCAGAGCT
TCTTAAAACACTAGAAACAGATGACGTGCAATTGCATGAAGCAACACTTAGAGCCCTA
ATTCAAATCAAGAGTGATCAATGCTCAAGCTCACTCATTAACTACCTTGACCGAGATAT
TCCCAACAAACCAATAGAAGCGCTTATAGAAGCCTTAACAGAGCAAAGAATGTGGGAT
GTTTCAGAAAAGATCCAACCCTTTCTTAACGACAAATCAGAAAGGATCGCTGGCTCTG
CAGCAGCTTTTTTCTACAGCTACACCGGTGAGATGACCTATTTAAACAAAGTTATCTCA
CTTCTTGATCACCAGAATCGCTTCATCAGGCAATCCGCTGCATTCGACCTAGCCCGTA
TCGGAACAATCAAAGCAACAGATCCAATCCTGACTGCCAAGATCCCCAACAACGTCAA
GATGTTTGCCATAAAAGCCATACTCAATAAATCGCTCAGCCGAAGC
AATCAAGCAGATTCTATTCCAGATACCGACCTCGCATCAATTCATTCCTCCCTCTTCAA
AGCACTTGACAGTCTCGCCAGAGACAACTTTTCGGGGAACCTATTGATTGAGCAAGA
CAACCAAATTCCAGAAACCTATCCAGGAGACGGCTCAACAGAGAGCGATCTACTTTCA
AATGCATTCGACAACCTAAGGTCACCATCCTTGACGAGCAGAAAATCAGG
CATAAAACAACTCGTCCGTGGTGCTAATCGTTTCAAAATCGATCTTCTTGATCTGTACT
TCTCAGAATCAGATCAAGACATCACAATGGGGCTGATCAAGGCCATGGCTGAACTCA

FIG. 22 cont.

AAAATCCCCATTACGCAAACGCACTTATTGATGCTATTGGGGTCGAAATTGGCAATCA
TTGCCAAGGAAACATTCGACGCGTCGCAGCGTGCGCCCTTGGTGACATCAATTGGAA
CGCGAAGATTTCGTCGCAATCACTGCATGCCGTTTTCAACAAACTCAAATGGACACTT
CATTCACCTGAAGACTGGGGTTTGCGCTACAGCGCATGCTTGGCGCTGGAAGGAATT
GGCAATGCCGATTCGATTAAACTCTTAAATGAAGCTAAAGCAAAAGAAACAGATCCAG
TCCTCTCTGCACGCCTTGACAAGGCAATACTAAAATCAAAAAATAAGACTTCTATCCAT
CACATCGAAAACAAAAAAGTTCTCTAAAACTAATGATAAAAGTATTGTTCGTCTGCCTC
GGACTAGTCATCGAGCTAGCAAGCTTGGCCGGATCCGGCCGGATCCGGAGTTTGTA
GAAACGCAAAAAGGCCATCCGTCAGGATGGCCTTCTGCTTAATTTGATGCCTGGCAG
TTTATGGCGGGCGTCCTGCCCGCCACCCTCCGGGCCGTTGCTTCGCAACGTTCAAAT
CCGCTCCCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAA
AACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCC
CTACTCTCGGTACCCGTCGGCTTGAACGAATTGTCGATCTCGGCTTGAACGAATTGTT
AGGTGGCGGTACTTGGGTCGATATCAAAGTGCATCACTTCTTCCCGTATGCCCAACTT
TGTATAGAGAGCCACTGCGGGATCGTCACCGTAATCTGCTTGCACGTAGATCACATA
AGCACCAAGCGCGTTGGCCTCATGCTTGAGGAGATTGATGAGCGCGGTGGCAATGC
CCTGCCTCCGGTGCTCGCCGGAGACTGCGAGATCATAGATATAGATTTCACTACGCG
GCTGCTCAAACTTGGGCAGAACGTAAGCCGCGAGAGCGCCAACAACCGCTTCTTGGT
CGAAGGCAGCAAGCGCGATGAATGTCTTACTACGGAGCAAGTTCCCGAGGTAATCGG
AGTCCGGCTGATGTTGGGAGTAGGTGGCTACGTCTCCGAACTCACGACCGAAAAGAT
CAAGAGCAGCCCGCATGGATTTGACTTGGTCAGGGCCGAGCCTACATGTGCGAATGA
TGCCCATACTTGAGCCACCTAACTTTGTTTTAGGGCGACTGCCCTGCTGCGTAACATC
GTTGCTGCTGCGTAACATCGTTGCTGCTCCATAACATCAAACATCGACCCACGGCGT
AACGCGCTTGCTGCTTGGATGCCCGAGGCATAGACTGTACAAAAAAACAGTCATAAC
AAGCCATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGG
ACCAGTTGCGTGAGCGCATACGCTACTTGCATTACAGTTTACGAACCGAACAGGCTTA
TGTCAACTGGGTTCGTGCCTTCATCCGTTTCCACGGTGTGCGTCACCCGGCAACCTT
GGGCAGCAGCGAAGTCGAGGCATTTCTGTCCTGGCTGGCGAACGAGCGCAAGGTTT
CGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGCTGTTCTTCTACGGCAAGGTGC
TGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAATTCACATACGCGGCCGCCTG
GGCCTTGAGCTCGAATTT

FIG. 22 cont.

SEQ ID NO: 9 - includes or encodes paraup1, *pebA*, *pebB*, and the hygromycine resistance cassette (inserted into neutral site 3 of *Syn* 7942 strain MX2064)

AAGCTTGCATGCCTGCAGGTGGCGCGCCATCGCTAGGACAGCTTAACCGCCACGTC
ATTGACTTTTTCTTCACAACCTGCGCGAAATTCAGAGGGCGATGCACCAGTGCACTTC
TTGAAAACTCGCGAGAAATACAACTGGTCATCAAACCCGACATTGCGGCCAACAGTA
GCGATGGGCATCCGCGTGGTGGACAAGAGCAGCTTGGCCTGCGAAATCCGTTGATC
TTCGCGCCAAGACAGGACCGAGATACCGAGCTGTTGGCGAAACAGGTGGCTCAAGC
GCGACGGGCTGAGACAAACATGTTGCGCGACGGACGCGATGTCGAAATTGCTGTCA
GCCAGATGATCGCTGATGTACTGGCATGCTTCGCGGACTCGGTTATCCATCGGCGGG
TGGAGCGACTCGTTGATGGCTTCCATCCGCCGCAACAGGAGTTGTTCCAACAGATTG
ATAGCGAGGAGTTCAGAGTAACGGCCCTCGCCTTGGCCGGCGTTAATAATCTGGCCA
AACAGATCACTAAAGTGTGGTTGATGGGCTTCATCGGGGCGAAAAAAACCCGTATTG
GCAAAAATAGAAGGCCAGTTCAACCATTCGTGCCAGTAAGCCCGTGGCCGAAAGTAA
ACCCACTGGTGATACCATTCGCGGGCCTCGGGATGGCGACCGTAGTGATGAATTTCA
CCAGGTGGGAACAGCAAAATGTCACCGGGGCGACAAACAAACTCCCGCCCTTGGTTT
TTGACAACGCCCTGACCACGAATCGTCAAGTTCAAAATATAGCCCTTCATACCCAAAG
GTCGATCGATGAAGAAATCCAAATACCCATTGGCTTCGATAGGTGTCAGGCCGGCCA
CGAGATGGGCATTAAAACTATAACCCGGCAACAAAGGATCATTCTGAGCTTCGGCCA
TACTTTTCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACAT
TGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCCCGCTTA
TTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAG
TGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGC
TATGCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCA
ACTCGTATAATGTTTCTCCATGTCGACAGGAAACAGGAATTACATATGTTTGATCCGTT
TCTTGAGGAATTACAAACTGGAATTCAAGCCCGCGGTGGCATATCAGTTGAAGTTCCG
GCCGGGCTGGAACACAATCAATCCCAGAAGGGCTCAAGCACCATCCAAAGCTGGCTT
TGGCAGGTTCCAGGTTTTCGTCGCTGGCGCGTCACCCGACTTGATGCAGGTGACAG
CCTTCAAGTTCTGAATTCCGTCGCATATCCCGATTTCGATTTGGACCATCCTTTGATG
GGTGTTGATCTGCTCTGGTTTGGCGCACGTCAAAAGCTAGTTGCGGTTCTTGATTTTC
AACCACTGGTTCAAGACAAAGACTATCTCGATCGTCATTTTGATGGTCTGAAAGATCT
GAATGCTCGTTTCCCGGATCTAAACGGAGAAGAAACGATGCGATCTTTCGATCCGAAT
CAATACTTCTCATCATGGCTACTTTTTTGCCGTGGAGGTTCTGAAGAGGCTGACAGGT
CACTGCCAAAGGCCTTCAGCGCCTTTTTGAAAGCCTATTGGGGTTTACACGATGAGG
CTTCCAAGGAACCATCCTCAATCTCACCTGGAGATGTGGAACGGCTTCAGAACGCCT
ACGACGTGTACAGCGCCGAGCGTGATCCTGCCCATGGATTGTTCACCAGCCATTTCG
GCAAGGAGTGGTCTGACCGGTTCCTGCACGAATTCCTTTTCCCTGCCAGTCAGCCCG
CATGAGCATTGATCTCCGCGCGTCGAGCCTTGATCCCGTTCAGATTCCGGGGTGGCG
ATGGCAGCCCTTTCTCGATGAAGCCAGTGCTGCACTCAAGCCGTTCAACCCGTCTCC
CTATCCCATAGCGGAAACGTTTCTGCAAAAGGAGGGCAGCACCGGTTCAAAAGCGAA
ACCCGTTCCGGTGACAACGGCGACCTGGGCCTGTTCCACAGACAAGTTGCGTCAGG
TGCGTTGTGCCTGCGTTGAAGCGGGTATGGCTGCTTCGGTGCTCAATTTTGTGATCA
ACCCGAGCTGTCGGTTCGACCTGCCGTTCTTCGGAGCCGATCTG
GTGACGCTTCCAAACGGCCATTTGCTCGCTCTGGATCTTCAACCGGTTGACAAGGCT
GATCCCGATCACACCCAACCCGTGTGGGAGCGACTGATGCCGTTGTTCGAGCGCTG
GCAAGCCGAACTCCCCGATGGAGGCCCCATCCCAGAAGAAGCCCAACCCTATTTCTC

FIG. 22 cont.

ACCGGCGTTTCTCTGGACCCGCATCCCGCTTGGGGAGGAGGGGGATGAACTGATTG
AACGCGTGATCCGCCCGGCCTTCATCGACTATCTGCAGCTTTACCTCAACCTCGTGG
CTGAAGCGGAACCCGTGTCTGACGACCGTGCGGAATTGCTCCTTTCAGGCCAAAAGC
GCTACACCGCGTATCGCGCCGAGAAGGATCCAGCCCGCGGCATGTTGACGCGGTTC
TACGGGAGCGAGTGGACAGAGTCGTACATCCATGGCGTGCTGTTCGATCTCGAGGA
CGCCGCTTAAACTAGTCATCGAGCTAGCAAGCTTGGCCGGATCCGGCCGGATCCGG
AGTTTGTAGAAACGCAAAAAGGCCATCCGTCAGGATGGCCTTCTGCTTAATTTGATGC
CTGGCAGTTTATGGCGGGCGTCCTGCCCGCCACCCTCCGGGCCGTTGCTTCGCAAC
GTTCAAATCCGCTCCCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACA
ACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTG
GCAGTTCCCTACTCTCGGTACCCGTCGGCTTGAACGAATTGTTAGACACTATTCCTTT
GCCCTCGGACGAGTGCTGGGGCGTCGGTTTCCACTATCGGCGAGTACTTCTACACA
GCCATCGGTCCAGACGGCCGCGCTTCTGCGGGCGATTTGTGTACGCCCGACAGTCC
CGGCTCCGGATCGGACGATTGCGTCGCATCGACCCTGCGCCCAAGCTGCATCATCG
AAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATAC
GCCCGGAGCCGCGGCGATCCTGCAAGCTCCGGATGCCTCCGCTCGAAGTAGCGCGT
CTGCTGCTCCATACAAGCCAACCACGGCCTCCAGAAGAAGATGTTGGCGACCTCGTA
TTGGGAATCCCCGAACATCGCCTCGCTCCAGTCAATGACCGCTGTTATGCGGCCATT
GTCCGTCAGGACATTGTTGGAGCCGAAATCCGCGTGCACGAGGTGCCGGACTTCGG
GGCAGTCCTCGGCCCAAAGCATCAGCTCATCGAGAGCCTGCGCGACGGACGCACTG
ACGGTGTCGTCCATCACAGTTTGCCAGTGATACACATGGGGATCAGCAATCGCGCAA
ATGAAATCACGCCATGTAGTGTATTGACCGATTCCTTGCGGTCCGAATGGGCCGAAC
CCGCTCGTCTGGCTAAGATCGGCCGCAGCGATCGCATCCATGGCCTCCGCGACCGG
CTGCAGAACAGCGGGCAGTTCGGTTTCAGGCAGGTCTTGCAACGTGACACCCTGTG
CACGGCGGGAGATGCAATAGGTCAGGCTCTCGCTGAATTCCCCAATGTCAAGCACTT
CCGGAATCGGGAGCGCGGCCGATGCAAAGTGCCGATAAACATAACGATCTTTGTAGA
AACCATCGGCGCAGCTATTTACCCGCAGGACATATCCACGCCCTCCTACATCGAAGC
TGAAAGCACGAGATTCTTCGCCCTCCGAGAGCTGCATCAGGTCGGAGACGCTGTCGA
ACTTTTCGATCAGAAACTTCTCGACAGACGTCGCGGTGAGTTCAGGCTTTTTCATTGT
TTAACTTTGTTTTAGGGCGACTGCCCTGCTGCGTAACATCGTTGCTGCTCCATAACAT
CAAACATCGACCCACGGCGTAACGCGCTTGCTGCTTGGATGCCCGAGGCATAGACT
GTACCCCAAAAAAACAGTCATAACAAGCCATGAAAACCGCCACTGCGCCGTTACCAC
CGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTGAGCGCATACGCTACTTGCATT
ACAGCTTACGAACCGAACAGGCTTATGTCCACTGGGTTCGTGCCTTCATCCGTTTCCA
CGGTGTGCGTCACCCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCCT
GGCTGGCGAACGAGCGCAAGGTTTCGAATTCACATACGCGGCCGCCTGGGCCTTGA
GCTCGAATT

FIG. 22 cont.

SEQ ID NO: 10 - deleted *cpcE* sequence (deleted from *Syn* 7942 mutant strain MX2064)

ATGAGTGAAGCGGGGACGCCAGCTGAGGCGCTGACCTACGAACAAGCGATCGCGAA
TCTCCGACAGACAGCAGATACGGGCGATCGCTACTACGCAGCTTGGTGGCTGGGTC
GGTTTCGGATGAAGCAGCCAGAGGCGATCGCGCTGTTGATTGAAGCCTTAGATGATA
GCCTCGATCGCGCACCTGATGGCGGCTATCCCCTACGGCGCAATGCCGCACGCGCA
TTGGGAAAACTGGAAAGTCCTGAGGCGATCGCACCGTTGATTGCCTGCTTGCAGTGC
GAGGACTACTACGTTCGCGAGGCTGCAACCCAGTCCTTAGGTGAGTTGCAAGCCACA
GTTGCGGTTCCAGCGTTATTGAAACTGTTAGAGGGCGGACCTGAGGCGATCGCCGC
GATTCCGGGTAAACCCCATCTGACTCAGCCAGCGGATGCGGTGATGGAAACCCTGG
GACAACTGCGAGCAACGGTTGCTGTCCCTGTGGTGCAAGCGTTTCTGGAGCATCCGA
TCGATAAAATTCGCCTAGCAGCCGCACGATCGCTCTATCAGCTCACCGGCGACGATC
ACTATGCTGAGCGGGTTGTTCAAGGTTTGAGTGACCCGAAATTACAGCGCCGGCGGT
CGGCCCTGATGGATTTAGGGGCGATCGGCTATTTGCCCGCTGCACCGCAAATTGCCC
AGACGCTTGCCGAGAATAGTCTCAAACTGATCTCGCTCAAAGGGCTGCTCGATACTC
ATCTGCGGCAACAGACCCCCGAGGCGATCGCAGAGTTGGATGAGTCGGCAATCGCG
CTGATGGATTTGATGGATGGTTTGCTGTAG

SEQ ID NO: 11 – sequence replacing deleted SEQ ID NO: 10; includes the kanamycin resistance cassette

TTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAA
TACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTT
CCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAAT
ACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAG
TGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTC
AACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTC
ATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTA
CAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTT
TCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAG
TGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAG
GCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAAC
GCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGA
TAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAAT
CAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTTGAATATG
GCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATG
ATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGGCTTT

FIG. 22 cont.

SEQ ID NO: 57 – includes or encodes Ptrc, cpcAB genes with the cpcA gene encoding the Y130C mutation, and a kanamycin resistance cassette (inserted into neutral site 2 of *Syn* 7942 strain MX2479)

CGCGCCATCGCTTGCAATTCGCGCTAACTTACATTAATTGCGTTGCGCTCATTGACCA
CTCTCCAAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTATCGGCCAGAGCACGC
GGGGAGGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCGTTTCACCAGCGACAC
GGGCAGCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAGTTGCAACAGTCGATC
CACGGACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGTGGTCAGAGGCGGGAT
ATAACAACTCGAATCTTCAGTATCATCATAGCCCACGACACTAATATCTGCGCCGACG
CGGAGGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGCCATCTGGTCATTCGC
GACCAGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTGCATTGTTTGCTGGAA
ACCCGACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGGTTGGATCTGATTGCG
GGTGAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGCACTCACGCTGGAGAGCG
GGCCTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGACCAGATGCTCCACACCC
AAGCGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAATGGGGGTTTGATCGGAG
ACGTCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCTTCAACAGCGATAGCATC
TTGGTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACACGCTGAGCCAGGAGGTT
GTGGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCAACCATGGAGACGACCAC
GCTTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCAACAATTTGACTCGGGGC
GTGCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAAGCTCTGTTTTCCGGCCA
GCTGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGCCATCGCAGCCTCGACT
TTTTCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAACCACGCGACTGACAGTTT
GGTAGCTCACACCTGCGTATTCTGCAACATCATACAGCGTGACTGGCTTCACATTGAC
CATCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCAC
CATTCGATGGTGTCAACGTACGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCA
GCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTC
GCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCATAACGGTTCT
GGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGG
AATTGTGAGCGGATAACAATTGTCGACAGGAAACAGGAATTACATATGACTTTTGATG
CTTTCACCAAGGTGGTGGCACAAGCCGATGCCCGTGGCGAATTTTTGAGCGACGCCC
AACTGGACGCGCTGAGCCGCTTGGTTGCAGAAGGCAACAAACGGATTGATACGGTCA
ACCGCATCACCGGTAATGCTTCGTCGATCGTCGCTAACGCAGCGCGTGCATTGTTTG
CAGAGCAACCTTCTCTGATTGCTCCTGGCGGCAACGCATACACGAACCGTCGGATGG
CGGCTTGTCTGCGCGACATGGAAATCATTCTCCGCTACGTGACCTACGCGGTCTTCA
CCGGCGATGCTTCCATTCTCGACGACCGCTGTTTGAACGGTCTGCGTGAGACCTACT
TGGCTCTGGGCGTGCCCGGTGCATCGGTGGCAGAAGGCGTTCGCAAGATGAAAGAC
GCAGCTGTGGCGATTGTGAGCGACCGCAACGGCATCACCCAAGGTGACTGTTCAGC
GATCATTTCCGAGCTGGGCAGCTACTTCGACAAAGCTGCTGCTGCAGTTGCCTAGTC
ATCGACTGGGATTGAGATAACAGACCTTTTTTCAGAGAAATAGGGAATCATGTCCAAG
ACTCCTCTGACCGAAGCTGTCGCTGCTGCTGATTCGCAAGGGCGTTTTCTGAGCAGC
ACTGAACTGCAAGTTGCATTTGGTCGTTTCCGTCAAGCTGCTTCTGGTTTGGCAGCG
GCTAAGGCGTTGGCAAACAATGCTGACAGCTTGGTCAACGGTGCA
GCGAACGCTGTTTACAGCAAGTTCCCCTACACCACCAGCACGCCTGGCAACAACTTT
GCATCGACGCCGGAAGGCAAAGCGAAGTGTGCGCGTGACATTGGTTACTATCTGCG
GATTGTGACCTATGCATTGGTTGCGGGTGGCACGGGTCCGATTGATGAGTACCTGTT

FIG. 22 cont.

GGCAGGTCTTGATGAGATCAACAAGACCTTCGACTTGGCGCCGAGCTGGTGTGTGGA
AGCGCTGAAGTACATCAAAGCGAATCATGGCTTGAGTGGTGACTCTCGCGATGAAGC
CAACTCCTACATCGACTACCTCATCAATGCCCTCAGCTAGActagtcAtcgagctagcaagcttgg
ccggatccggccggatccggagtttgtagaaacgcaaaaaggccatccgtcaggatggccttctgcttaatttgatgcctggca
gtttatggcgggcgtcctgcccgccaccctccgggccgttgcttcgcaacgttcaaatccgctcccggcggatttgtcctactcag
gagagcgttcaccgacaaacaacagataaaacgaaaggcccagtctttcgactgagcctttcgttttatttgatgcctggcagtt
ccctactctcGGTACCCGTCGGCTTGAACGAATTGTCAAGTCAGCGTAATGCTCTGCCAGT
GTTACAACCAATTAACCAATTCTGATTAGAAAAACTCATCGAGCATCAAATGAAACTGC
AATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAA
GGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCG
ATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTT
ATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTT
ATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCA
CTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACG
CGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAAC
ACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAA
TGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGAT
AAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATC
TCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCG
CATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCG
AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTaGAG
CAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGC
AGACAGTTTTATTGTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATT
TTGAGACACAACGTGGCTTTcGAACGAGCGCAAGGTTTCGAATTCacatacGCGGCCGC
ctgggccttgagctcgaatt

FIG. 22 cont.

SEQ ID NO: 58 – includes or encodes Ptrc, cpcAB genes with the cpcA gene encoding the A86K mutation, and a kanamycin resistance cassette (inserted into neutral site 2 of *Syn* 7942 strain MX2507)

CGCGCCATCGCTTGCAATTCGCGCTAACTTACATTAATTGCGTTGCGCTCATTGACCA
CTCTCCAAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTATCGGCCAGAGCACGC
GGGGAGGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCGTTTCACCAGCGACAC
GGGCAGCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAGTTGCAACAGTCGATC
CACGGACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGTGGTCAGAGGCGGGAT
ATAACAACTCGAATCTTCAGTATCATCATAGCCCACGACACTAATATCTGCGCCGACG
CGGAGGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGCCATCTGGTCATTCGC
GACCAGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTGCATTGTTTGCTGGAA
ACCCGACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGGTTGGATCTGATTGCG
GGTGAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGC
ACTCACGCTGGAGAGCGGGCCTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGA
CCAGATGCTCCACACCCAAGCGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAA
TGGGGGTTTGATCGGAGACGTCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCT
TCAACAGCGATAGCATCTTGGTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACA
CGCTGAGCCAGGAGGTTGTGGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCA
ACCATGGAGACGACCACGCTTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCA
ACAATTTGACTCGGGGCGTGCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAA
GCTCTGTTTTCCGGCCAGCTGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGC
CATCGCAGCCTCGACTTTTTCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAAC
CACGCGACTGACAGTTTGGTAGCTCACACCTGCGTATTCTGCAACATCATACAGCGT
GACTGGCTTCACATTGACCATCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATA
CCGCGAAAGGTTTTGCACCATTCGATGGTGTCAACGTACGACTGCACGGTGCACCAA
TGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAAT
CACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGC
CGACATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCC
GGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTGTCGACAGGAAACAGGAA
TTACATATGACTTTTGATGCTTTCACCAAGGTGGTGGCACAAGCCGATGCCCGTGGC
GAATTTTTGAGCGACGCCCAACTGGACGCGCTGAGCCGCTTGGTTGCAGAAGGCAA
CAAACGGATTGATACGGTCAACCGCATCACCGGTAATGCTTCGTCGATCGTCGCTAA
CGCAGCGCGTGCATTGTTTGCAGAGCAACCTTCTCTGATTGCTCCTGGCGGCAACGC
ATACACGAACCGTCGGATGGCGGCTTGTCTGCGCGACATGGAAATCATTCTCCGCTA
CGTGACCTACGCGGTCTTCACCGGCGATGCTTCCATTCTCGACGACCGCTGTTTGAA
CGGTCTGCGTGAGACCTACTTGGCTCTGGGCGTGCCCGGTGCATCGGTGGCAGAAG
GCGTTCGCAAGATGAAAGACGCAGCTGTGGCGATTGTGAGCGACCGCAACGGCATC
ACCCAAGGTGACTGTTCAGCGATCATTTCCGAGCTGGGCAGCTACTTCGACAAAGCT
GCTGCTGCAGTTGCCTAGTCATCGACTGGGATTGAGATAACAGACCTTTTTTCAGAGA
AATAGGGAATCATGTCCAAGACTCCTCTGACCGAAGCTGTCGCTGCTGCTGATTCGC
AAGGGCGTTTTCTGAGCAGCACTGAACTGCAAGTTGCATTTGGTCGTTTCCGTCAAG
CTGCTTCTGGTTTGGCAGCGGCTAAGGCGTTGGCAAACAATGCTGACAGCTTGGTCA
ACGGTGCAGCGAACGCTGTTTACAGCAAGTTCCCCTACACCACCAGCACGCCTGGCA
ACAACTTTGCATCGACGCCGGAAGGCAAAGCGAAGTGTGCGCGTGACATTGGTTACT
ATCTGCGGATTGTGACCTATGCATTGGTTGCGGGTGGCACGGGTCCGATTGATGAGT

FIG. 22 cont.

ACCTGTTGGCAGGTCTTGATGAGATCAACAAGACCTTCGACTTGGCGCCGAGCTGGT
GTGTGGAAGCGCTGAAGTACATCAAAGCGAATCATGGCTTGAGTGGTGACTCTCGCG
ATGAAGCCAACTCCTACATCGACTACCTCATCAATGCCCTCAGCTAGActagtcAtcgagcta
gcaagcttggccggatccggccggatccggagtttgtagaaacgcaaaaaggccatccgtcaggatggccttctgcttaatttt
gatgcctggcagtttatggcgggcgtcctgcccgccaccctccgggccgttgcttcgcaacgttcaaatccgctcccggcggat
ttgtcctactcaggagagcgttcaccgacaaacaacagataaaacgaaaggcccagtctttcgactgagcctttcgttttatttg
atgcctggcagttccctactctcGGTACCCGTCGGCTTGAACGAATTGTCAAGTCAGCGTAATGC
TCTGCCAGTGTTACAACCAATTAACCAATTCTGATTAGAAAAACTCATCGAGCATCAAA
TGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTC
TGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTAT
CGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTAT
TAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACT
GAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCC
AGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGA
TTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGG
AATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGA
ATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGT
AACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATT
CCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTT
GCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTC
GCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCA
TGTTGGAATTTAATCGCGGCCTaGAGCAAGACGTTTCCCGTTGAATATGGCTCATAAC
ACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTT
ATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGGCTTTcGAACGAGCGC
AAGGTTTCGAATTCacatacGCGGCCGCctgggccttgagctcgaatt

SEQ ID NO: 59 – includes or encodes Ptrc, CpeS1, and a blasticidin resistance (inserted into neutral site 5 of *Syn* 7942 strain MX2505)

agcttgcatgcctgcaggtGGCGCGCCATCGCTTGCAATTCGCGCTAACTTACATTAATTGCGT
TGCGCTCATTGACCACTCTCCAAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTA
TCGGCCAGAGCACGCGGGGAGGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCG
TTTCACCAGCGACACGGGCAGCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAG
TTGCAACAGTCGATCCACGGACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGT
GGTCAGAGGCGGGATATAACAACTCGAATCTTCAGTATCATCATAGCCCACGACACTA
ATATCTGCGCCGACGCGGAGGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGC
CATCTGGTCATTCGCGACCAGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTG
CATTGTTTGCTGGAAACCCGACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGG
TTGGATCTGATTGCGGGTGAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGCACT
CACGCTGGAGAGCGGGCCTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGACCA
GATGCTCCACACCCAAGCGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAATGG
GGGTTTGATCGGAGACGTCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCTTCA
ACAGCGATAGCATCTTGGTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACACGC
TGAGCCAGGAGGTTGTGGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCAACC
ATGGAGACGACCACGCTTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCAACA
ATTTGACTCGGGGCGTGCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAAGCT

FIG. 22 cont.

CTGTTTTCCGGCCAGCTGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGCCAT
CGCAGCCTCGACTTTTTCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAACCAC
GCGACTGACAGTTTGGTAGCTCACACCTGCGTATTCTGCAACATCATACAGCGTGACT
GGCTTCACATTGACCATCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGC
GAAAGGTTTTGCACCATTCGATGGTGTCAACGTACGACTGCACGGTGCACCAATGCT
TCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACT
GCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGAC
ATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCT
CGTATAATGTGTGGAATTGTGAGCGGATAACAATTGTCGACAGGAAACAGGAATTACA
TATGTTTCCCCTCCAGTCATATCCACCAATGACGATGGTGGATTTTTTCGAAGCAAGC
CGTGGGACCTGGTTGAACCGACGTGCTGTTCATCATTTGGATCACCAGGATGATGAA
GCAGCAGATTCTAATCTTGTTATCGAACCATTTAAAAATGATGATCCGGCAGTTCGCA
GCATTTGCGAAGCCCTAAACATCAACATGATCGACAGTACTGGTGGAGCTAGATTTTG
GTGGGAAAGTAATATTAAAAAAGGAGTCCGCAACGAAGATTATGCTGCTGTTGTCATC
GATGTACCCAACCGAGATAATGCTCGAAAGGGTTTCTTACTACGAGATGTAGGATATG
TTGAAAAGCAGGCGGTATTGAGCACTTACGTTTTTGCCGAAGATGGCGTGTTGACGA
TCACTACAAGATATGACACGAATATTGGAATTGAACGATGCTGGTTTGTGACTGATCA
GATCCGAATGCGTGTCAGTTCTGTCCAATGCTTGGATGGTGTCGCAATGACTACCTAC
TGCACTGAATTTCGCTGTCCAACAGATGCTGATATCAATGCCATATCTGAGCATGCCA
GGCAGATCGCTCGTTCGACTGCATCTATTGGAGCTTAAActagtcAtcgagctagcaagcttggcc
ggatccggccggatccggagtttgtagaaacgcaaaaaggccatccgtcaggatggccttctgcttaatttgatgcctggcagt
ttatggcgggcgtcctgcccgccaccctccgggccgttgcttcgcaacgttcaaatccgctcccggcggatttgtcctactcagg
agagcgttcaccgacaaacaacagataaaacgaaaggcccagtctttcgactgagcctttcgttttatttgatgCCTGGCA
GTTCCCTACTCTCGGTACCCGTCGGCTTGAACGAATTGTTAGACACTAACCTTCCCAA
ACATAGCCACTAGGGAGCAGCTCCCGGATGCCAACAGCCGTGGGCTGGCCATCGCT
ATCTTTCACAATTGCTTTGATGCCGGGGTGCAGATCCAGGAGCACCTGCCGGCAACG
GCCACAGGGGCTCAAGATCCCGCGGTTTTCGTTGCCGATGGCAACGATGCACGTCA
GGTTGCCGGCTGCAGCCGCTGCTGCAGTACCCAGGACGACCAGTTCCGCACAAGGG
CCACCGGTAAAATGGTAGACATTCACACCGGTAAAGATGCGGCCATCGCTCGACAAT
GCAGCCGACGCCACGCTATAATCTTCGCTAATAGGAATCGAGTTAATAGTCGCGGTC
GCGCGTTCGATCAGTGTAGACTCCTCTTGACTGAGGGGTTTCGCCATGGTTTAGTTC
CTCACCTTGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACGCG
GCCGCCTGGGCCTTGAGCTCGAATT

SEQ ID NO: 60 – includes or encodes Ptrc, CpeS2, and a blasticidin resistance cassette (inserted into neutral site 5 of *Syn* 7942 strain MX2506)

ATCGCTTGCAATTCGCGCTAACTTACATTAATTGCGTTGCGCTCATTGACCACTCTCC
AAACGGCTCACTTGCCGTGCCAGCTGCATGAGACTATCGGCCAGAGCACGCGGGGA
GGCCGTTTGCGTGTTTGGCGCCAAGGTGGTTTTGCGTTTCACCAGCGACACGGGCA
GCAGCTGATTTCCTTTAACTGCCTGCCCTTGGCTCAGTTGCAACAGTCGATCCACGG
ACGTCTGACCCAACAAGCGGAAATCTTGCTTGATCGTGGTCAGAGGCGGGATATAAC
AACTCGAATCTTCAGTATCATCATAGCCCACGACACTAATATCTGCGCCGACGCGGA
GGCCGCTCTCCGTGATCGCACGCATCGCGCCCAAAGCCATCTGGTCATTCGCGACC
AGCATGGCCGTAGGCACGATGCCTTCATTCAGCATTTGCATTGTTTGCTGGAAACCC

FIG. 22 cont.

GACATAGCGCTCCAATCGCCCTCGCGCTCGGCGATTGGTTGGATCTGATTGCGGGT
GAGGTATTTATGCCAGCCCGCGAGTCGCAGGCGAGCACTCACG
CTGGAGAGCGGGCCTGCCAAGAGAGCAATCTGCTGATGGCCCAGCGCGACCAGATG
CTCCACACCCAAGCGTGTACCGTCCTCGTGCGAGAAGATGATGCTATTAATGGGGGT
TTGATCGGAGACGTCCAGGAACAACGCGGGAACGTTCGTGCAGGCCGCTTCAACAG
CGATAGCATCTTGGTCATCCAGCGGGTAGTTGATAATCAGGCCCGACACACGCTGAG
CCAGGAGGTTGTGGACCGCAGCTTTGCAAGCTTCCACGCCACTCCGTTCAACCATGG
AGACGACCACGCTTGCCCCCAGTTGATCCGCACGCGATTTAATCGCGGCAACAATTT
GACTCGGGGCGTGCAGCGCGAGAGAGCTCGTGGCAACCCCGATCAACAAGCTCTGT
TTTCCGGCCAGCTGCTGCGCGACGCGGTTGGGGATATAATTCAGCTCGGCCATCGC
AGCCTCGACTTTTTCACGCGTCTTTGCCGACACATGGGAGGCTTGATTAACCACGCG
ACTGACAGTTTGGTAGCTCACACCTGCGTATTCTGCAACATCATACAGCGTGACTGGC
TTCACATTGACCATCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAA
AGGTTTTGCACCATTCGATGGTGTCAACGTACGACTGCACGGTGCACCAATGCTTCT
GGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGC
ATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACAT
CATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCG
TATAATGTGTGGAATTGTGAGCGGATAACAATTGTCGACAGGAAACAGGAATTACATA
TGAGCACAATATTAAAAAGTATGACGATTGAGCAATTTGTTGCTCAAAGTGTGGGTAA
ATGGCGCTCCATGAGATCAGGCCATTCTCTCGCTTTTCAACAATTTGAAGACGTTCTT
AGCGAAGTAATTATTGAATCCATCGAGAAAGACGATTCTGCTGTTCAAGATTTACTCTC
AACCGCAACTTCTAACCAAGGACATAGCTCCGACATCGTCGCGCCATTCAGGATGGA
ATGGTCAGCTGAAAGTGACTGGGAGCCCGAAGATCCATCTCAAGTTTCATCAGGCTC
GTGCCTGATCATCCCACTGAAAAAAAATGATTATTCTGGCATCTTGATCAGAAGTGTG
GGGTATGCTGAATCCGAATTAGCAGAGTCGACATACCAGTTTTTAGACGATGGCACAT
TCTTGCTTACAACGCATTATGAGCAATCAATGGCAGAGGAAAGAATCTGGTTTGTTTC
AGACAATGTTCGGTGCAGATCATCTGTATTGAAGACATCTGCAGGCTCAGGAGTTCTA
CAAACTTCATTTGCCTCTGAAGTCAGACGAGTCAAGGCCTAGActagtcAtcgagctagcaagc
ttggccggatccggccggatccggagtttgtagaaacgcaaaaaggccatccgtcaggatggccttctgcttaatttgatgcct
ggcagtttatggcgggcgtcctgcccgccaccctccgggccgttgcttcgcaacgttcaaatccgctcccggcggatttgtccta
ctcaggagagcgttcaccgacaaacaacagataaaacgaaaggcccagtctttcgactgagcctttcgttttatttgatgCCT
GGCAGTTCCCTACTCTCGGTACCCGTCGGCTTGAACGAATTGTTAGACACTAACCTTC
CCAAACATAGCCACTAGGGAGCAGCTCCCGGATGCCAACAGCCGTGGGCTGGCCAT
CGCTATCTTTCACAATTGCTTTGATGCCGGGGTGCAGATCCAGGAGCACCTGCCGGC
AACGGCCACAGGGGCTCAAGATCCCGCGGTTTTCGTTGCCGATGGCAACGATGCAC
GTCAGGTTGCCGGCTGCAGCCGCTGCTGCAGTACCCAGGACGACCAGTTCCGCACA
AGGGCCACCGGTAAAATGGTAGACATTCACACCGGTAAAGATGCGGCCATCGCTCGA
CAATGCAGCCGACGCCACGCTATAATCTTCGCTAATAGGAATCGAGTTAATAGTCGC
GGTCGCGCGTTCGATCAGTGTAGACTCCTCTTGACTGAGGGGTTTCGCCATGGTTTA
GTTCCTCACCTTGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAA
CGCGGCCGCCTGGGCCTTGAGCTCGAATT

FIG. 22 cont.

SEQ ID NO: 12 – consensus amino acid sequence for the PCB attachment site in phycocyanin

(A/S)(K/A)C(I/L/A)RD

SEQ ID NO: 13 – consensus PUB attachment site

(A/S)(K/A)CSRD

SEQ ID NO: 14 - PcpcB

TGAGAAAAAGTGTAAACAAATATTAAGAAAAAGATCAGAAAAATTTAACAACACGTAAT
AAAAAAATGCGTCACTACGGGTTATAAATTTACATGAAAGGTTAAAACACTTTTCTGAG
ACGATTTTGATAAAAAAGTTGTCAAAAAATTAAGTTTCTTTACAAATGCTTAACAAAAAC
TTGGTTTTAAGCACAAAATAAGAGAGACTAATTTGCAGAAGTTTTACAAGGAAATCTTG
AAGAAAAAGATCTAAGTAAAACGACTCTGTTTAACCAAAATTTAACAAATTTAACAAAA
CAAACTAAATCTATTAGGAGATTAACTAAGC

SEQ ID NO: 15 - PggpS

CTTGAAAAAGTTGAGGTATTAATAGAGCTTGATAAATGATAATAAAAACAGATTTAGCT
CTTATTTTAAGGGAAAAAGAAATAAATAAAATATTAGTAAATATCAAAAATATCAGCCTT
TCAAAAATAATTTGACTCTTTTCAAAAAAAAATGTTATCTTTAAGGTATGCTTTAAACCT
TAAATACTTCTATTGGTAACACTGTTCTCAATCTTATTTCAGATTTTCCCATTGAGCATA
AATAAAATATTAAGCAGAAGTAGAAAAGGTTGATATTAGCAATAATAAAAATTAACAAT
AAAATGTGAAAACAGATTACTACTGATTATTTATTGCCATGAGCTAATTAGTAATAATTT
GTCTTTTTTGATCGAAAAATGAAATTTTTTAAGCGGAGGAACTGAAAATTA

SEQ ID NO: 16 – PirtA

TAGAGTATGATAAAATGACAAGGAAAGGATTATTTTCTCTTGTTTAAATTCTCAAGATT
CTTATGCTTATTTATTTTATGTAAGTGTCTCTTTTCCTTGAAATAGAAAGAAAAAAGTGG
CTAATTTTGAGAAAAGCTAACAACGCTTTGGTTAACTAAAAATCAAAAGTGAGATTACT
GATCGCTTAAGAAATGGAGTATTGATT

SEQ ID NO: 17 - PnblA

GCAGTTAGATAAATAAGTAATGAGCGGGAGAAATAGGGGCAAATGGCCATTCGCCCC
TACAGGGAGGTGGCAGGTGTTAGGGTGTTTAGGGGATGAGGTGATGAGGGTAGAGG
GAGATAAGGTGTCGGGTTTCAGATTTCAGGTTTTAGAAGAAAGTAACGAGTAATTATC
AACTATTCACTATTCACTATTGCCTGTTGCCCTTCTCTCCTTGAAATATAAAAAAATGTA
AAAATATCATTAAGAAAAGTAACAAAATAAACAGAAAGGTTGACAAAGTTGACGCTTTA
ATATCCGTATGTTAGCTTTATAACAACGAAATCAACGGAGGAGTGAAA

FIG. 22 cont.

SEQ ID NO: 18 - PpetJ

TATTTATATATAAACTCGAATAAAATTATCAATATAAAGTCAAACTATATCTATCCTATTT
TAACTGCTATTGGTAAGTCCCTTAATTAGTGTTGGGGTGAATAGATTTTAAAAGGGCA
AACCCCCCTTTATCCTCCCTCGAGAGGGGGGAGGGCAAAAGGCAAGGGGCAAGGGA
AAAATTAAGAATTAAGAATTAAAAACTCCGAACACCTGTAGGGGCGAATAGCCATTCG
CTTCCCCTCATCCCCCCATCTCCCCAACACCCTAAGCCCCTACTCGTTACTCATTTAT
TTACATCATTTATTTACATCATTAAGAAAAGTAACAAATTTTGACAAGTAGTCTTTTGAC
AGGAAAAAGCAAATTCTCGAAGATGAAAACAATAGAAAAAAATTCAATCTTACAGTAAC
G

SEQ ID NO: 19 - PmrgA

AGAGTTATATTTACATAGTGTGTGCGAGTAAGGGCAACTTTTGTAGGTAGATGAATAA
ACCTCAAATTACTCATCTTAAAAGACGATATTTTTAATCTATTCTTCTGTAATAAAATAC
TTCTTTCGATAGAGATATTTAATACTTTTGAGAGATGAAAATAATTTCAATAATTGTCAT
GATAGAGAGTAAGTGCAAATAAGAAAAAATTGATTT

SEQ ID NO: 20 - PppsA

GTGATATTTGGTTTATTCTATATTTTCCTTAAGTAAAAATTCAGTCATGAGGGAAACTTT
TGTTAAAATTTGCTTTAAATTAATAGGAAGATCATTAAGAAAATCTTAAAAAGATTGAGT
TTTTAGATCGAAATTATTGAAGAAAAATTAACAGGGGTTCTGCTCAAAATTTTATTAAAT
TACTCTACTGTAGTAAAGGAGAAATTTTATT

SEQ ID NO: 21 - PpstS

ATAACCAATGGGACTTGAATTTTAGATCCATTTATTTAATTCTATTTTTGTTACATTTCTT
TATATTAATCAGAATTATGTTACTTTGTTTTGTTTTATGTCGTTACCTTATTGAAGAAAG
AGTGGATGAGAAGGTAAATGACGGGGCATAAATATCGATTCGTTGTCAGAATAAGCT
GTTTTATTCACTTAACTGGTTGTTTGCCAATTTCTCCCTAATTCCCATAACTTGTATAAC
TAAATTTAATAATCAATTTTAGTAAATTAAGAATAGGTTAAAAGTAGTATTTAGAATTAA
GTTAACTTTAATAAATTTCCTGTATTTTTTTATAGAAAAAAGTATAAAATAAAAACATATC
AAAAAAGTTTGAAATGACAAT

FIG. 22 cont.

SEQ ID NO: 22 - PrnpA

GAATAGTTGATAATTACTCGTTACTCATTACTCACTTAAACCTGCCACCTGATACCTGC
CACCTCTCCCCCCATCACCTCATCCCCTCAACATTCCGAACCCCTTGACACTTTGAAC
TAAAATTGTATTAAAGTGCAAATCTGGACGGGGTTAACCAGTGTGACTTATAATAGTAA
ACGCTGTTTTTTATAATAAATAAGCTAAATATTTAAAAACTATGAGTAAATATACACTAA
ATGGTACTAGACGTAAGCAGAAAAGAACCTCCGGTTTCCGCGCCCGTATGAGAACCA
AAAATGGTAGAAAAGTAATTCAAGCTCGTCGTAATAAGGGTAGAAAAAGATTAGCAGT
ATAAAATTACTGTTAAATAAGGAAGCTAAGTTTAGCATTTTAAGTTTGATATTACTAATC
ATTAAATTTACTGTGAAATATAGGTGGGACTACCATCAAAGCATCGACTGAAACGGCG
TTTAAATTTCCAATCTGTTTATCAACAGGGTATTCGCCGCTCTAGTCGTTATTTTATTGT
CCGAGGGTTACGG

SEQ ID NO: 23: - Generalized PnirA sequence

5'$(n)_{116}$ATGCAAAAAACGAAT$(n)_7$ATGTGTAAAAAGAAA$(n)_{15}$GTAGTCAAAGTTAC$(n)_{22}$TA
ATGT$(n)_{55}$CCGAGGACAAA$(n)_2$ATG-3'

SEQ ID NO: 24 - Generalized PnirA sequence with nucleotide changes in the RBS

5'$(n)_{116}$ATGCAAAAAACGAAT$(n)_7$ATGTGTAAAAAGAAA$(n)_{15}$GTAGTCAAAGTTAC$(n)_{22}$TA
ATGT$(n)_{55}$GGAGGATCAGCC$(n)_2$ATG-3'

SEQ ID NO: 25 - Generalized PnirA sequence with nucleotide changes in the operator region and the TATA box

5'$(n)_{116}$ATGCAAAAAACGCAT$(n)_7$ATGCGTAAAAAGCAT$(n)_{15}$GTAATCAAAGTTAC$(n)_{22}$TA
ATAT$(n)_{55}$CCGAGGACAAA$(n)_2$ATG-3'

SEQ ID NO: 26 - Generalized PnirA sequence with nucleotide changes in the RBS, the operator region and the TATA box

5'$(n)_{116}$ATGCAAAAAACGCAT$(n)_7$ATGCGTAAAAAGCAT$(n)_{15}$GTAATCAAAGTTAC$(n)_{22}$TA
ATAT$(n)_{55}$GGAGGATCAGCC$(n)_2$ATG-3'

SEQ ID NO: 27 - Co^{2+}-inducible PcorT

CAT$(n)_7$GTTTACTCAAAACCTTGACATTGACACTAATGTTAAGGTTTAGGCT$(n)_{15}$CAAGT
TAAAAAGCATG

SEQ ID NO: 28 - modified variant of PcorT includes changes in the RBS

CAT$(n)_7$GTTTACTCAAAACCTTGACATTGACACTAATGTTAAGGTTTAGGCT$(n)_{15}$GAGG
ATAAAAAGCATG

FIG. 22 cont.

SEQ ID NO: 29 - modified variant of PcorT includes changes in the TATA box

CAT$(n)_7$GTTTACTCAAAACCTTGACATTGACTAATGTTAAGGTTTAGAAT$(n)_{15}$CAAGTTA AAAAGCATG

SEQ ID NO: 30 - modified variant of PcorT includes changes in the RBS and the TATA box

CAT$(n)_7$GTTTACTCAAAACCTTGACATTGACACTAATGTTAAGGTTTAGAAT$(n)_{15}$GAGGA TAAAAACCATG

SEQ ID NO: 31 - Zn^{2+}-inducible PsmtA

$(n)_8$AATACCTGAATAATTGTTCATGTGTT$(n)_4$TAAAAATGTGAACAATCGTTCAACTATTT A$(n)_{12}$GGAGGT$(n)_7$ATG

SEQ ID NO: 32 - Zn^{2+}-inducible PsmtA with changes in the RBS

$(n)_8$AATACCTGAATAATTGTTCATGTGTT$(n)_4$TAAAAATGTGAACAATCGTTCAACTATTT A$(n)_{10}$AAGGAGGTGAT$(n)_4$ATG

SEQ ID NO: 33 - Zn^{2+}-inducible PsmtA with changes in the RBS

$(n)_8$AATACCTGAATAATTGTTCATGTGTT$(n)_4$TAAAAATGTGAACAATCGTTCAACTATTT A$(n)_{10}$AAGGAGGTAT$(n)_5$ATG

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/41384

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 1/12, 1/13, 9/88, 15/63; C07K 14/195, 14/00 (2016.01)
CPC - C12N 1/12, 1/13, 9/88, 15/63; C07K 14/195, 14/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12N 1/12, 1/13, 9/88, 15/63; C07K 14/195, 14/00 (2016.01)
CPC: C12N 1/12, 1/13, 9/88, 15/63; C07K 14/195, 14/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C12N 1/12, 1/13, 9/88, 15/63; C07K 14/195, 14/00 (text search)
USPC: 435/232, 320.1, 252.3; 530/350

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Patents; Google Scholar
Search terms: Phycobiliproteins, light harvesting antenna proteins, PebA (15,16 dihydrobiliverdin oxidoreductase), PebB,, RcpG,CpcA, CpcB, PUB (phycourobilin), exogenous, recombinant, Cyanobacteria, Synechococcus, increased photosynthetic activity or efficiency

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y ---- A	2013/0171677 A1 (BRYANT et al.) 4 July 2013 (04.07.2013). Especially para [0091], [0287], [0288], [0302], [0304], [0354], sheet 5 fig 6.	1, 15-17, 20, 22, 23, 26, 29, 38, 52, 53, 67 ---------- 2, 3, 9, 18, 19, 27, 54, 55, 59
Y		---------- 5, 7, 24, 30, 57, 61
Y	US 2003/0104379 A1 (LAGARIAS et al.) 5 June 2003 (05.06.2003). Especially SEQ ID NO: 46, 51	2, 3, 18, 19, 54, 55
Y	UNIPROT entry P00312. C-phycocyanin-1 beta chain. [online]. 24 June 2015 [retrieved 17 November 2016]. Available on the internet: <URL: http://www.uniprot.org/uniprot/P00312.txt?version=110 >. Especially pg 2.	9, 27, 59
A	US 2008/0301839 A1 (RAVANELLO) 4 December 2008 (04.12.2008). Especially SEQ ID NO: 10679	5, 24, 57
A	UNIPROT entry P00308. C-phycocyanin-1 alpha chain. [online]. 24 June 2015 [retrieved 17 November 2016]. Available on the internet: <URL: http://www.uniprot.org/uniprot/P00308.txt?version=102> Especially pg 2	7, 30, 61
A	KIRST et al. Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. Biochim Biophys Acta October 2014 Vol 1837 No 10 Pages 1653-1654. Especially abstract, pg 1655 fig 1, fig 2; pg 1659 fig 7.	1

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 November 2016	15DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/41384

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NO: 1-3, 61, 63

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/41384

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-3, 5, 7, 9, 15-20, 22-24, 26, 27, 29, 30, 38, 52-55, 57, 59, 61, 67 limited to SEQ ID NOs: 1-3, 61, 63

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/41384

----continuation of Box III (Lack of Unity of Invention)----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-38, 52-67, drawn to a modified Cyanobacteria comprising insertion of one or more exogenous genes to increase photosynthetic activity or the production of phyocyanin.

The modified Cyanobacteria will be searched to the extent that the exogenous nucleotide sequences encompasses nucleotide sequences encoding PebA (SEQ ID NO: 2), PebB (SEQ ID NO: 3), RpcG (SEQ ID NO: 1), CpcB (SEQ ID NO: 63) and a variant CpcA (SEQ ID NO: 61). It is believed that claims 1-3, 5, 7, 9, 15-20, 22-24, 26, 27, 29, 30, 38, 52-55, 57, 59, 61, 67 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 1-3, 61, 63. Additional exogenous genes will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected exogenous genes(s). Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: PebA (SEQ ID NO: 1), PebB (SEQ ID NO: 2), RpcG (SEQ ID NO: 1), CpeS (SEQ ID NO: 64), deletion of CpcE (SEQ ID NO: 10)(claims 1-3, 5, 11-20, 22-24, 32, 33, 35-38, 52-55, 57, 63-67).

Group II+: Claims 39-51, drawn to a method to increase photosynthetic activity in Cyanobacteria by generating a functioning non-native PEB. Group II+ will be searched upon payment of additional fee(s). The method may be searched, for example, to the extent that the method involves inserting exogenous nucleotide sequences encoding PebA (SEQ ID NO: 4) and PebB (SEQ ID NO: 5). It is believed that claims 39, 40, (50, 51)(in part), read on this exemplary invention. Additional exogenous polynucleotide(s) will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be searched/examined. An exemplary election would be the exogenous polynucleotide comprises RpcG (SEQ ID NO: 7), CpcB (SEQ ID NO: 68) and a variant CpcA (SEQ ID NO: 66) (claims 39-44, (50, 51)(in part)).

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ has the special technical feature of a Cyanobacteria composition comprising one or more exogenous nucleic acids, not required by Group II+

Group II+ has the special technical feature of a method on increasing in Cyanobacteria photosynthetic activity, not required by Groups I+

Among the inventions listed as Groups I+ and II+ are the specific exogenous nucleotide sequences of PebA, PebB, RpcG, CpcB, a variant CpcA, CpeS, CpcE, recited therein. Each of the inventions of Group I+ and II+ requires a unique set of exogenous nucleotide sequences, not required by the other inventions.

Common technical features:

Groups I+ and II+ share the common technical feature of a Cyanobacteria comprising exogenous nucleotide sequences of PebA and PebB.

Group I+ has the special technical features of claims 1, 16 and 52.

Group II+ has the special technical feature of claim 39.

However, said common technical features do not represent a contribution over the prior art, and is obvious over US 2013/0171677 A1 to BRYANT et al. (hereinafter "Bryant").

------continued on next sheet-----

-----continued from previous sheet-----

As to claim 1, Bryant teaches a modified Cyanobacteria comprising: (1) insertion of one or more exogenous nucleotide sequences encoding PebA, PebB (para [0302]; "PEB [i.e. phycoerythrobilin; see sheet 5 fig 6 for synthetic pathway] can readily be attached to CpcA by CpcE/CpcF lyase when all necessary proteins are heterologously expressed in E. coli. This observation prompted a study to determine if this phenomenon could be replicated in a cyanobacterium, in which chromophorylation might be more tightly controlled because of the potential to cause deleterious effects on the photosynthetic capacities of cells. For this experiment, pebA and pebB, encoding a 15,16 dihydrobiliverdin oxidoreductase and the phycoerythrobilin reductase, respectively, from the chromatically acclimating cyanobacterium Synechococcus sp. strain PCC 7335 were placed under the control of the strong cpcBA promoter of Synechocystis sp. strain PCC 6803 and introduced into a neutral site on the endogenous pAQ1 plasmid of Synechococcus sp. strain PCC 7002"). Bryant does not specifically teach the modified Cynaobacteria having increased photosynthetic activity as compared to a Cyanobacteria of the same species without the modification. However, Bryant does teach that PEB produced by PebA and PebB associate with the alpha subunit of phycocyanin (i.e. CpcA) (para [0304]; "In order to examine the distribution of PEB among the major PBPs [i.e. pigmented phycobiliprotein] isolated from PBS [i.e. phycobilisomes] isolated from the pebA pebB and wild-type strains, PBPs were separated by analytical SDS-PAGE alongside aliquots of purified recombinant [His]6-CpcA carrying PCB or PEB.....When the absorbance profiles of the major PBP components of PBS isolated from the pebA pebB overexpression strain (FIG. 9C) were compared to those from the wild-type strain (FIG. 4B), the only PBP subunit that exhibited a major difference in absorption was the phycocyanin alpha subunit") is a part of the light-harvesting antenna that broadens the light absorption spectrum available for photosynthesis (para [0287]; "Most cyanobacteria employ light-harvesting antennae known as phycobilisomes (PBS) to collect light that is not efficiently absorbed by chlorophyll (Chl) for photosynthesis. PBS are, multi-subunit, supramolecular structures composed of both pigmented phycobiliproteins (PBPs) and usually non-pigmented linker proteins. Four different linear tetrapyrrole chromophores (bilins): phycocyanobilin (PCB), phycoerythrobilin (PEB), phycoviolobilin (PVB), and phycourobilin (PUB) can be bound to cyanobacterial PBPs"). Bryant further teaches exogenous CpcB (para [0091], and RpcG (para [0354]). Consequently, it would have been obvious for one of ordinary skill in the art to try and use standard means known in the art to measure increased photosynthetic activity, such as O2 release, in comparison to an unmodified Cyanobacteria of the same species, without undue effort, and a high probability of success.

As to claim 16, Bryant teaches modified Cyanobacteria wherein the modification results in utilization of a functioning non-native PEB (para [0302], [0304], sheet 5 fig 6). Bryant does not specifically teach increased photosynthetic activity in the modified Cyanobacteria as compared to a Cyanobacteria of the same species without the modification However, Bryant does teach that PEB produced by PebA and PebB associate with the alpha subunit of phycocyanin (i.e. CpcA) (para [0304]), is a part of the light-harvesting antenna that broadens the light absorption spectrum available for photosynthesis (para [0287]). Consequently, it would have been obvious for one of ordinary skill in the art to try and use standard means known in the art to measure increased photosynthetic activity, such as O2 release, in comparison to an unmodified Cyanobacteria of the same species, without undue effort, and a high probability of success.

As to claim 39, concerning a method to increase photosynthetic activity in a Cyanobacteria, Bryant teaches inserting one or more exogenous nucleotide sequences encoding PebA and PebB wherein expression of the PebA and PebB results in production of a functioning nonnative PEB (para [0302], [0304], sheet 5 fig 6). Bryant does not specifically teach increased photosynthetic activity [as compared to the same Cyanobacteria species without the modification]. However, Bryant does teach that PEB produced by PebA and PebB associate with the alpha subunit of phycocyanin (i.e. CpcA) (para [0304]), a part of the light-harvesting antenna that broadens the light absorption spectrum available for photosynthesis (para [0287]). Consequently, it would have been obvious for one of ordinary skill in the art to try and use standard means known in the art to measure increased photosynthetic activity, such as O2 release, in comparison to an unmodified Cyanobacteria of the same species, without undue effort, and a high probability of success.

As to claim 52, Bryant teaches a Cyanobacteria that naturally produces phycocyanobilin (PCB) wherein the Cyanobacteria is modified to produce functioning non-native PEB (para [0302], [0304]) and functioning nonnative PUB (i.e. phycourobilin) (para [0274]; "When the PecE/PecF lyase was produced together with HT-CpcA in a strain capable of synthesizing PEB (harboring pPebS; Table 1), the resulting E. coli cells were pale yellow-orange in color. Suggesting that a mixture of products was produced, the purified HT-CpcA protein from these cells had absorption maxima at 497 nm and at 560 nm. The former value is characteristic of PUB while the latter is similar to that observed for PEB as described above"), wherein the Cyanobacteria produces phycocyanin with a red pigment (para [0274]) and phycocyanin with a purple pigment (para [0288]; Thus, proteins incorporating PCB have the most red-shifted absorption maxima while those with PUB have the most blue-shifted maxima").

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The Inventions lack unity with one another.

Therefore, Group I+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 1(iii): will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.: 1-38, 52-67, limited to PebA (SEQ ID NO: 2), PebB (SEQ ID NO: 3), RpcG (SEQ ID NO: 1), CpcB (SEQ ID NO: 63) and a variant CpcA (SEQ ID NO: 61). (claims 1-3, 5, 7, 9, 15-20, 22-24, 26, 27, 29, 30, 38, 52-55, 57, 59, 61, 67).

Note concerning claim 23: Claim 23 is written to depend from itself. For the purposes of the International Search and Opinion, claim 23 is interpreted to depend from claim 22.