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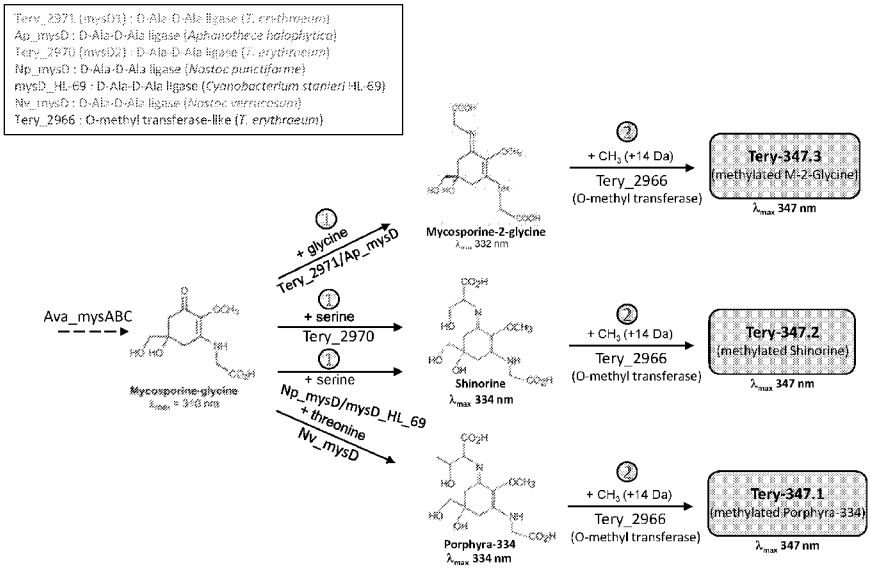

FIG. 10

(57) Abstract: Modified host cell and cyanobacterial cells that are capable of producing various mycosporine-like amino acids (MAAs), useful as natural UV absorbing compound, are described.

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TITLE

PRODUCTION OF MYCOSPORINE-LIKE AMINO ACIDS EMPLOYING ENHANCED PRODUCTION STRAINS AND NOVEL ENZYMES

REFERENCE TO SEQUENCE LISTING

[0001] This application contains a sequence listing submitted by EFS-Web, thereby satisfying the requirements of 37 C.F.R. §§ 1.821-1.825.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the recombinant production of known as well as novel mycosporine-like amino acids (MAAs) in cyanobacterial host cells and other host cells. MAAs have UV protective ability and can be used as natural skin protectants, such as sunscreens.

BACKGROUND OF THE INVENTION

[0003] Cyanobacteria can be utilized as bio-factories to produce compounds of interest from sunlight, CO_2, and nutrients. The transformation of the cyanobacterial genus *Synechococcus* with genes of interest has been described (U.S. Patent Nos. 6,699,696 and 6,306,639, both to Woods et al.). The transformation of the cyanobacterial genus *Synechocystis* has been described, for example, in PCT/EP2009/000892 and in PCT/EP2009/060526. The transformation of the cyanobacterial genus *Cyanobacterium* sp. has been described (U.S. Patent No. 8,846,369, U.S. Patent No. 9,315,832, and PCT/US2013/077364).

[0004] Penetration of UV radiation through the skin can damage the cellular DNA, induce genetic mutations and can lead to skin cancer. UV-A is regarded as the main cause of skin aging and wrinkling of human skin. Since both UV-A and UV-B are harmful, protection for both kinds of rays are recommended. The FDA has approved a list of active chemical/physical ingredients for use in sunscreens which absorb into the top layers of skin. For some people, however, the ingredients can lead to skin irritation, allergic reactions and even skin damage or aging. Further, the commonly used sunscreen component oxybenzone has recently been found to contribute to damage to coral reefs by bleaching of corals and disruption of coral reproduction and growth (Downs et al., "Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands", *Arch Environ Contam Toxicol.*, 70:265-88

(2016). The impacts of other chemical sunscreen substances on aquatic ecosystems are also of concern (Kim et al., "Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review" *Environ Int.*, 70:143-157 (2014). Further, the use of nanoparticles, such as zinc oxide and titanium dioxide, are also controversial. A good alternative sunscreen product or skin care product component would be a natural UV-absorbing substance.

[0005] Mycosporine-like amino acids (MAAs) are natural, water-soluble, carbonaceous, nitrogenous compounds that absorb light in the UV-A/UV-B range between 310 and 362 nm.

[0006] MAAs are naturally produced by a number of organisms, including certain algae species, cyanobacteria, dinoflagellates and corals. MAAs apparently act as natural sunscreens in these organisms. In addition to their ability to act as a sunscreen, MAAs also have antioxidant activity (Wada et al. "Mycosporine-Like Amino Acids and Their Derivatives as Natural Antioxidants" *Antioxidants* 4:603-646 (2015)).

[0007] Several cyanobacteria naturally synthesize MAAs such as mycosporine-glycine, shinorine, porphyra-334, mycosporine-2-glycine, palythine, asterina, mycosporine-glutamic acid-glycine, mycosporine-ornithine, usujirene, and palythene (for a review, see Carreto et al., "Mycosporine-Like Amino Acids: Relevant Secondary Metabolites", *Chemical and Ecological Aspects Mar Drugs*, 9:387-446 (2011)).

[0008] Some species of cyanobacteria have been found to naturally produce MAAs, and can be cultivated in a sustainable manner for the production of desired ultraviolet screening compounds. Currently, MAA-based bio-sunscreen compounds are exclusively sourced from marine macroalgae, such as *Porphyra umbilicalis*, containing MAAs such as porphyra-334 and shinorine. Purified MAAs are not currently commercially available, but extracts from the red alga *Porphyra umbilicalis* are sold and used in cosmetic products (Helioguard® and Helionori®). The *Porphyra* extracts are reported to contain the MAA porphyra-334 and shinorine with absorption coefficients (ε molar) of 42,300 and 44,700 at 334 nm. The concentration of the MAAs in the extract is low: in the range of 1 % of total dry weight (Hartmann et al. "Quantitative analysis of mycosporine-like amino acids in marine algae by capillary electrophoresis with diode-array detection", *Jour. Pharm. Biomed. Analysis*, 138:153-157 (2017)).

[0009] However, cyanobacteria naturally produce lower amounts of MAAs (0.03 to 0.98 mg MAA/g dry cell weight) compared to *P. umbilicalis* (10 mg/g dry cell weight), complicating the

industrial benefit of cyanobacteria (Garcia-Pichel et al., "The phylogeny of unicellular, extremely halotolerant cyanobacteria", *Archives of Microbiology*, 169:469–482 (1998).

[0010] Further, induction of the production of naturally occurring MAAs in cyanobacteria often occurs in response to certain types of stress, such as abiotic stresses and UV radiation, making the commercial production of MAAs with algae or cyanobacteria difficult (Singh et al., "Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/screening compounds", *Indian J Exp Biol.* 46:7-17 (2008)).

[0011] Due to these problems, there is a constant need to develop novel routes for the synthesis of MAA compounds. There is also a need to identify novel MAA compounds having new absorption characteristics different from known mycosporine-like amino acids.

SUMMARY OF THE INVENTION

[0012] Provided herein are modified host cells, in particular cyanobacterial host cells, plasmid constructs, and methods to produce various MAAs from cyanobacterial cultures.

[0013] One embodiment of the present invention is directed to a genetically modified cyanobacterial cell for the production of mycosporine-glycine exhibiting a low pigment phenotype compared to the wildtype with respect to the chlorophyll and phycocyanin content of the cell, comprising

- at least two copies of a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO:6 or the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70,
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO**:** 9, or the third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC of HL69**).** Such a genetically modified cyanobacterial cell produces a higher amount of mycosporine glycine due to the fact that it exhibits

a low pigment phenotype having a reduced content of chlorophyll and phycocyanin in the cell compared to the respective wildtype cyanobacterial cell. Such a reduced content of the antenna complexes of the cyanobacteria ensures that cells with lower amount of pigment do not shade other cells to a great extent, so that the incident light can penetrate deeper into a cell culture and more light can reach the cells. Additionally, the second copy of the gene mysA also ensures that higher amount of the first intermediate compound desmethyl-4-deoxygadusol is present in the cyanobacterial cell leading to an increase in the production of the final MAA Mycosporine-glycine, which is produced from desmethyl-4-deoxygadusol by the further enzymatic actions of MysB and MysC.

[0014] Another embodiment of the present invention is directed to a genetically modified cyanobacterial cell for the production of a mycosporine-like amino acid precursor 4-deoxygadusol, comprising

-a fusion recombinant gene encoding a fusion protein including first and second enzymes involved in the production of a mycosporine-like amino acid (MAA) in one polypeptide chain, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 (MysA Ava_3858) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (MysA-HL69), the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6, (MysB Ava_3857) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (MysB -HL69),

-a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,

-a second recombinant gene that encodes a second recombinant enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava_3857) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (MysB_HL-69). Such a genetically modified cyanobacterial cell is also able to produce a higher amount of the precursor 4-deoxygadusol due to the "*substrate channeling effect*" of the MysAB fusion protein. The presence

of genes coding for single proteins MysA and MysB in the genetically modified cyanobacterial cell ensures that the formation of multimeric protein complexes does not lead to the formation of aggregates of multiple MysAB fusion proteins, which either might be less active or completely inactive. The genes coding for single proteins MysA and MysB can form for example dimers with the MysAB fusion protein so that a dimer can be formed between one MysAB fusion protein and one single MysA protein, which as shown in the experimental data section is still enzymatically active.

[0015] A further embodiment of the present invention is directed to a genetically modified host cell for the production of a mycosporine-like amino acid Tery-347, comprising

-a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,

- a second recombinant gene that encodes the second recombinant enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70,

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9, or the third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC of HL69).

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 or the sixth recombinant gene encoding a sixth enzyme having at least 80% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase),

- a further recombinant gene encoding a further enzyme, wherein the further gene is selected from a group consisting of the following genes:

a) a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Tery_2971, MysD1), or the fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 (Ap_MysD from *Aphanothece*),

b) a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 52 (Tery_2970, MysD2), or the fourth gene having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 76 (Nv_MysD from *Nostoc verrucosum*), or the fourth gene having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12. (NpF5597) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 74 (MysD_HL-69). Such a genetically modified host cell is able to produce a novel MAA compound called Tery-347, which has an absorption maximum at a wavelength of 347 nm. In particular, if all possible further genes are present in the genetically modified host cell, such a host cell can produce the different novel MAA compounds Tery-347.1, Tery-347.2 and Tery-347.3 as described herein.

[0016] Another embodiment of the present invention is directed to a genetically modified cyanobacterial host cell, wherein the O-methyltransferase Tery_2966 can be replaced with other enzymes having low sequence homology of 49% to 60% with Tery_2966. Furthermore, the invention is also directed to a genetically modified cyanobacterial host cell wherein the clavaminic acid synthetase-like enzyme Tery_2972 can be replaced with enzymes having a low sequence homology of 60 to 65 % to Tery_2972. A further variant of the present invention is directed to genetically modified cyanobacterial host cells, wherein the nonribosomal peptide synthetase Tery_2968 is replaced with enzymes having a low sequence homology of 40% to 55% to Tery_2968.

DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1 is a general biosynthetic pathway for the synthesis of several MAAs in cyanobacteria. The main enzymes involved in the various conversion steps are indicated above the arrows for each individual step. In general, the enzymes MysA, MysB, and MysC catalyze the conversion of sedoheptulose-7-phosphate to mycosporine-glycine. Various different MysD enzymes attach different amino acids to mycosporine-glycine resulting in different MAAs, in particular shinorine, porphyra-334, mycosporine-2-glycine and mycosporine-glycine-alanine. In particular, a first gene encoding for a first enzyme, MysA catalyzes the conversion of sedoheptulose-7-phosphate to desmethyl-4-deoxygadusol. This compound is subsequently converted to 4-deoxygadusol catalyzed by the enzyme family MysB, which is a second enzyme

encoded by a second gene. Further, 4-deoxygadusol is converted to mycosporine-glycine via the attachment of a glycine catalyzed by the enzyme family MysC, a third enzyme encoded by a third gene. Starting with mycosporine-glycine different enzymes can be employed resulting in the synthesis of different MAA compounds. In particular, a fourth gene encoding a fourth enzyme, the so-called enzyme family D-alanine-D-alanine ligase "MysD2" can catalyze the formation of shinorine/porphyra-334 through the attachment of either serine or threonine to mycosporine-glycine. Some of the enzymes in this MysD2 enzyme family catalyze the attachment of both serine and threonine to mycosporine-glycine resulting in a mixture of shinorine and porphyra-334 (i.e. D-alanine-D-alanine ligase MysD_HL-69 [*Cyanobacterium stanieri* HL-69]). The enzyme marked "Nv_mysD" (D-alanine-D-alanine ligase MysD [*Nostoc verrucosum* KU005]) catalyzes mainly the attachment of threonine to mycosporine-glycine resulting in porphyra-334 (shinorine/porphyra-334 ratio around 10 to 90 for Nv_MysD) while the enzyme marked "Tery_2970" (D-alanine-D-alanine ligase MysD2 [*Trichodesmium erythraeum* IMS101]) catalyzes mainly the attachment of serine to mycosporine-glycine resulting in the formation of shinorine (shinorine/porphyra-334 ratio around 90 to 10 for Tery_2970). Another member of this enzyme family of fourth enzymes, "Np_MysD" (NpF5597 of *Nostoc punctiforme)* not just catalyzes the formation of shinorine/porphyra-334, but also the attachment of alanine to mycosporine-glycine resulting in mycosporine-glycine-alanine. Another D-alanine-D-alanine ligase enzyme family called "MysD1" catalyzes the attachment of glycine to mycosporine-glycine resulting in mycosporine-2-glycine. This enzyme family of fifth enzymes is encoded by fifth genes. Members of this "MysD1" enzyme family are for instance Ap_MysD (also referred to as "Ap3855" from *Aphanothece halophytica*) and Tery_2971 (D-alanine-D-alanine ligase MysD1 from *Trichodesmium erythraeum* IMS101). In the following, the denomination of the various enzyme families as first, second, third, fourth, fifth etc. enzymes encoded by the respective genes described in Fig. 1, will be used throughout the description of the invention**.** Table 1B also explains the nomenclature employed for the various genes and their respective enzymes for MAA production.

[0018] Fig. 2A to 2C are graphs representing the growth (FIG. 2A), mycosporine-2-glycine production (FIG. 2B), and the carbon partitioning (FIG. 2C) of low pigment strains AB4102 and AB4111 in comparison to the reference strain AB1334 in GC vial experiments.

[0019] Fig. 3A to 3D depict the cell growth (FIG. 3A), the mycosporine-2-glycine production (FIG. 3B), the carbon partitioning (FIG. 3C) and the whole cell absorbance spectra (FIG. 3D) of

the two low pigment strains AB 4102 and AB4111 in comparison to the reference strain AB1334 in LvPBR cultivations.

[0020] Fig. 4A to 4C depict the graphs for the growth (FIG. 4A), the shinorine/porphyra-334 production (FIG. 4B) and the carbon partitioning (FIG. 4C) of the low pigment strain AB4101 in comparison to the reference strain AB4094 conducted in GC vial experiments.

[0021] Fig. 5A to 5D show the cell growth (FIG. 5A), the shinorine production (FIG. 5B), the carbon partitioning (FIG. 5C) and the whole cell absorbance spectra (FIG. 5D) of the low pigment strain AB4101 in comparison to the reference strain AB4094 in LvPBR cultivations.

[0022] Fig. 6A to 6D show the cell growth (FIG. 6A), the shinorine production (FIG. 6B), the carbon partitioning (FIG. 6C), and the whole cell absorbance spectra (FIG. 6D) for the low pigment strain AB4103 in comparison to the reference strain AB4068 in LvPBR experiments.

[0023] Fig. 7A and 7B depict the MAA content in the biomass (FIG. 7A) and the cell associated MAA fraction (FIG. 7B) over a time period of 25 days of cultivation for the low pigment strain AB4103 in comparison to the reference strain AB4068. Fig. 7C and 7D depict the MAA content in the biomass (FIG. 7C) and the cell associated MAA fraction over a time period of 25 days of cultivation (FIG. 7D) for the low pigment strain AB4101 in comparison to the reference strain AB4094.

[0024] Fig. 8A and 8B show the general design of a MysAB fusion protein, and the "peptide linker X" (SEQ ID NO: 82) and "peptide linker Y" (SEQ ID NO: 84) located between the proteins MysA and MysB.

[0025] Fig. 9A to 9C show the cell growth (FIG. 9A), the shinorine production (FIG. 9B), and the carbon partitioning (FIG. 9C) for the strains AB4179 and AB4181 both expressing a MysAB fusion protein in comparison to the reference strain AB4100.

[0026] Fig. 10 shows proposed pathways for the synthesis of the novel, not yet known MAA compounds Tery-347.1, Tery-347.2 and Tery-347.3 employing the enzymes Tery_2966, Tery_2971, Tery_2970, Np_MysD, MysD_HL-69 and Nv_MysD. As already explained in relation to Fig. 1, mycosporine-glycine can be converted into shinorine or porphyra-334 via the attachment of either serine or threonine catalyzed by a fourth enzyme encoded by a fourth gene (Tery_2970, Np_MysD, Nv_MysD or MysD_HL-69). A fifth gene encodes a fifth enzyme including among others Tery_2971 or Ap_MysD to produce mycosporine-2-glycine via the attachment of glycine. These intermediate compounds shinorine/porphyra-334 or mycosporine-2-glycine can then be converted to the novel compounds Tery-347.1, Tery-347.2 or Tery-347.3 employing the enzyme

Tery_2966, which is a sixth enzyme encoded by a sixth gene. The denomination of a "*six gene encoding a sixth enzyme*" will in the following be used for the enzyme family including Tery_2966 and OMT_PCC7335 which catalyze the addition of a methyl group to shinorine/porphyra-334 or mycosporine-2-glycine.

[0027] Fig. 11 depicts the HPLC chromatogram of a reference strain AB1333, which produces mycosporine-glycine-alanine, porphyra-334 and shinorine.

[0028] Fig. 12 shows the HPLC chromatogram of the strain AB4105 producing a mixture of Tery-347.1 and Tery-347.2.

[0029] Fig. 13A shows the HPLC chromatogram of the strain AB4104 producing Tery-347.3. Fig. 13B shows the HPLC profile of the *E. coli* strain #3186 expressing Ava_MysABC, Tery_2966 and the non-ribosomal peptide synthetase NRPS_NIES2100 from *Calothrix* sp. NIES-2100, which produces a Tery-347 compound. It is not yet known whether this compound is anyone of the Tery-347.1, Tery-347.2 or Tery-347.3 compounds disclosed herein or a structurally different MAA compound also having an absorption maximum at 347 nm.

[0030] Fig. 14 shows the absorbance spectra of MAA extracts including Tery-322 for the strains AB4074, AB4075, AB4076 and AB4090.

[0031] Fig. 15A and 15B show the HPLC spectra of the strains AB4075 (FIG. 15A) and AB4076 (FIG. 15B) expressing O-methyltransferase-like enzymes having a low sequence homology to Tery_2966, both strains producing Tery-322.

[0032] Fig. 16 shows the HPLC profile of the ABCyano4 strain AB4140 expressing the low sequence homology enzyme for the enzyme Tery_2972.

[0033] Fig. 17 shows the gradient between the mobile phase A and mobile phase B employed during a hydrophobic interaction liquid chromatography (HILIC) run.

[0034] Fig. 18 depicts the plasmid map of the plasmid #2848.

[0035] Fig. 19 depicts the plasmid map of the plasmid #2865.

[0036] Fig. 20 depicts the plasmid map of the plasmid #2891.

[0037] Fig. 21 depicts the plasmid map of the plasmid #2892.

[0038] Fig. 22 shows the plasmid map of the plasmid #2995.

[0039] Fig. 23 shows the plasmid map of the plasmid #3050.

[0040] Fig. 24 depicts plasmid map of the plasmid #3075.

[0041] Fig. 25 depicts the plasmid map of the plasmid #3095.

[0042] Fig. 26 shows the plasmid map of the plasmid #3096.

[0043] Fig. 27 shows the plasmid map of the plasmid #3110.

[0044] Fig. 28 shows the plasmid map of the plasmid #3122.

[0045] Fig. 29 shows the plasmid map of the plasmid #3123.

[0046] Fig. 30 shows the plasmid map of the plasmid #3125.

[0047] Fig. 31 shows the plasmid map of the plasmid #3113.

[0048] Fig. 32 depicts the plasmid map of the plasmid #3140.

[0049] Fig. 33 shows the plasmid map of the plasmid #3190.

[0050] Fig. 34 depicts the plasmid map of the plasmid #3213.

[0051] Fig. 35 shows the plasmid map of the plasmid #3214.

[0052] Fig. 36 depicts the plasmid map of the plasmid #3130.

[0053] Fig. 37 shows the plasmid map of the plasmid #3131.

[0054] Fig. 38 shows the plasmid map of the plasmid #3186.

[0055] Fig. 39 shows the general proposed pathways for the production of the MAA compounds shinorine, mycosporine-methylamine-serine, palythine, mycosporine-2-glycine, Tery-322, Tery-364, Tery-347.2 and Tery-347.3. In addition to the enzyme families already presented in Fig. 1 and 10, Fig. 39 shows that a sixth enzyme encoded by a sixth gene, including Tery_2966 can also catalyze the addition of a methyl group to mycosporine-glycine resulting in another related mycosporine-glycine (Tery-322), which is a methylated mycosporine-glycine variant with a molecular weight of 259 Da and an absorbance maximum at 322nm. An eighth enzyme, encoded by the eighth gene, Tery_2968 further catalyzes the conversion of Tery-322 to the unknown MAA compound Tery-364, a MAA variant with a molecular weight of 356 Da and an absorbance maximum at 364nm. A seventh enzyme, encoded by a seventh gene, Tery_2972 catalyzes the conversion of mycosporine-2-glycine into palythine. A ninth enzyme encoded by a ninth gene, Tery_2969 catalyzes the decarboxylation of shinorine resulting in mycosporine-methylamine-serine or asterina-330.

[0056] Fig. 40 shows the plasmid map of one endogenous ABCyano4 plasmid, pABCyano4B, having an approximate copy number of 30-50 copies per cell in ABCyano4. The integration site for the plasmids used for transformation of ABCyano4 via homologous recombination is indicated by an arrow.

[0057] Fig. 41 shows the plasmid map of one endogenous ABCyano4 plasmid, pABCyano4C, having an approximate copy number of 12-20 copies per cell in ABCyano4. The integration site

for the plasmids used for transformation of ABcyano4 via homologous recombination is indicated by an arrow.

[0058] Fig. 42 shows the plasmid map of the plasmid #3094.

[0059] Fig. 43 shows the plasmid map of the plasmid #2991.

[0060] Fig. 44 shows the plasmid map of the plasmid #3182.

[0061] Fig. 45 shows the plasmid map of the plasmid #3183.

[0062] Fig. 46shows the plasmid map of the plasmid #3211.

[0063] Fig. 47 shows the plasmid map of the plasmid #3287.

[0064] Fig. 48 shows the plasmid map of the plasmid #3289.

DETAILED DESCRIPTION

[0065] Disclosed herein is a cyanobacterial organism that has been modified so that it is capable of producing large amounts of a mycosporine-like amino acid (MAA). The MAA so produced can be isolated and utilized for consumer products, such as sunscreens, skin treatments, and cosmetics.

[0066] Aspects of the invention utilize techniques and methods common to the fields of molecular biology, microbiology and cell culture. Useful laboratory references for these types of methodologies are readily available to those skilled in the art. See, for example, Molecular Cloning: A Laboratory Manual (Third Edition), Sambrook, J., et al. (2001) Cold Spring Harbor Laboratory Press; *Current Protocols in Microbiology* (2007) Edited by Coico, R, et al., John Wiley and Sons, Inc.; The Molecular Biology of Cyanobacteria (1994) Donald Bryant (Ed.), Springer Netherlands; *Handbook Of Microalgal Culture Biotechnology And Applied Phycology* (2003) Richmond, A.; (ed.), Blackwell Publishing; and "*The cyanobacteria, molecular Biology, Genomics and Evolution*", Edited by Antonia Herrero and Enrique Flores, Caister Academic Press, Norfolk, UK, 2008.

[0067] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Description of Embodiments of the Invention

[0068] One embodiment of the present invention is directed to a genetically modified cyanobacterial cell for the production of mycosporine-glycine exhibiting a low pigment phenotype compared to the wildtype with respect to the chlorophyll and phycocyanin content of the cell, comprising

- at least two copies of a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6, or the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (MysB of HL69**),**
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9, or the third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC of HL69**)**. Such a genetically modified cyanobacterial cell produces a higher amount of mycosporine glycine due to the fact that it exhibits a low pigment phenotype having a reduced content of chlorophyll and phycocyanin in the cell compared to the respective wildtype cyanobacterial cell. The low pigment phenotype with regard to chlorophyll is due to a Δycf37 gene inactivation associated with a low chlorophyll phenotype. The low phycocyanin phenotype is due to a promoter swap in the cpcBA operon. The native PcpcB promoter upstream of the cpcBA operon was replaced by the endogenous PoprB promoter of ABCyano4. The untranslated region of the cpcBA operon was retained in its native form. Such a reduced content of the antenna complexes of the cyanobacteria ensures that cells with lower amount of pigment do not shade other cells to a great extent, so that the incident light can penetrate deeper into a cell culture and more light can reach the cells. As a result the photoautotrophic cyanobacterial host cells can accumulate more biomass also leading to enhanced production of mycosporine-glycine and other related MAAs. Additionally, the second copy of the gene mysA, the first recombinant gene also ensures that higher amount of the first intermediate compound

desmethyl-4-deoxygadusol is present in the cyanobacterial cell leading to an increase in the production of the final MAA Mycosporine-glycine, which is produced from desmethyl-4-deoxygadusol by the further enzymatic actions of MysB, the second recombinant protein and MysC, the third recombinant protein.

[0069] Preferably, at least one, preferably two, most preferably all recombinant genes are operably linked to a promoter, which is inducible by the addition of an inducer, for example a change in metal-ion concentration. These promoters can preferably be promoters being inducible by the addition of zinc-ions, for example the PsmtA promoter or by the addition of copper-ions, for example the PpetE promoter.

[0070] According to a further embodiment of the genetically modified cyanobacterial cell, the cell further is for the production of shinorine and/or porphyra-334, comprising a fourth recombinant gene encoding of fourth enzyme, which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the 12 (NpF5597), 52 (Tery_2970), 74 (MysD [*Cyanobacterium stanieri* HL-69]), 76 (MysD [*Nostoc verrucosum* KU005]) or 78 (D-alanine-D-alanine ligase [*Actinosynnema mirum* DSM 43827]). This additional fourth recombinant gene encodes a fourth enzyme belonging to a heterogeneous group of so-called MysD2 enzymes, which can catalyze the conversion of mycosporine-glycine into shinorine and/or porphyra-334. In most cases these enzymes produce a mixture of shinorine and porphyra-334, except for the enzyme Nv_MysD (D-alanine-D-alanine ligase MysD [*Nostoc verrucosum* KU005]) and Tery_2970 (D-alanine-D-alanine ligase MysD2 [*Trichodesmium erythraeum* IMS101]).

[0071] A further variant of this genetically modified cyanobacterial cell can produce mycosporine-2-glycine and further comprises a fifth recombinant gene encoding a fifth enzyme D-alanine-D-alanine ligase enzyme family called "MysD1" which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO. 15 (Ap3855 from *Aphanothece halophytica*) and at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO. 50 (D-alanine-D-alanine ligase MysD1 from *Trichodesmium erythraeum* IMS101). These MAA producing enzymes can catalyze the attachment of glycine to mycosporine-glycine.

[0072] Another embodiment of the present invention is directed to a genetically modified cyanobacterial cell for the production of mycosporine-like amino acid precursor 4-deoxygadusol, comprising a fusion recombinant gene encoding a fusion protein including first and second enzymes involved in the production of a mycosporine-like amino acid (MAA) in one polypeptide chain, the first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence

identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant), the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 MysB variant or MysB_HL69 variant),

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant), and
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 MysB variant or MysB_HL69 variant). The fusion protein includes a fusion of the two enzymes MysA and MysB. Both enzymes can be separated by a short amino acid sequence in the fusion protein, for example the linker X or the linker Y as described herein. The MysA-MysB fusion protein ensures, that the product of the reaction catalyzed by MysA, desmethyl-4-deoxygadusol is formed in the proximity of the second enzyme MysB, which converts this compound into 4-deoxygadusol, resulting in a so-called "*substrate channeling effect*" increasing the amount of 4-deoxygadusol which is available for the synthesis of MAA compounds. The protein MysA is known to form dimers, so that the genetically modified cyanobacterial cell for the production of 4-deoxygadusol also includes a separate single first recombinant gene, coding for MysA and a separate single second recombinant gene, coding for MysB. This approach ensures that one single MysA protein can form a dimer-complex with one MysA-MysB fusion protein. This is supposed to avoid the formation of large aggregates of multimeric MysA-MysB fusion proteins, which might be less active or even inactive due to the large fusion proteins.

[0073] The genetically modified cyanobacterial cell including the gene for the MysAB fusion protein can furthermore include at least one third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 9 (Ava_3856 MysC), or the third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ

ID NO: 72 (MysC of HL69). This third recombinant protein, MysC catalyzes the attachment of glycine to 4-deoxygadusol and the formation of mycosporine-glycine. Due to the "*substrate channeling effect*" of the MysAB fusion protein, such as genetically modified cyanobacterial cell also produces more mycosporine-glycine in comparison to genetically modified cyanobacterial cells, which do not include a MysAB fusion protein, but which only harbor separate MysA, MysB, and MysC proteins.

[0074] Another embodiment of the genetically modified cyanobacterial cell including the gene coding for the MysAB fusion protein can additionally include a fourth recombinant gene encoding a fourth enzyme with at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to either one of the SEQ ID NO: 12, 15, 50, 52, 74, 76 or 78. These MysD proteins catalyze the attachment of glycine to mycosporine-glycine resulting in mycosporine-2-glycine or serine to mycosporine-glycine resulting in shinorine or the attachment of threonine to mycosporine-glycine resulting in porphyra-334. The enzyme Np_MysD can also attach alanine to mycosporine-glycine resulting in mycosporine-glycine-alanine. Furthermore non-ribosomal peptide synthetases such as Ava_3855 (SEQ ID NO. 18) can attach amino acids to mycosporine-glycine. Due to the presence of the MysAB fusion protein, such a genetically modified cyanobacterial cell also produces larger amounts of shinorine/porphyra-334 or mycosporine-glycine-alanine in comparison to other genetically modified cells lacking the MysAB fusion protein.

[0075] According to a further embodiment of this genetically modified cyanobacterial cell, the fusion recombinant gene encodes a linker with between 18 to 24 amino acids between MysA and the MysB. Such a linker is particularly suited to connect both MysA and MysB sections of the fusion protein, without interfering with the activity of either enzymes.

[0076] Another embodiment of the present invention is directed to a genetically modified host cell for the production of a mycosporine-like amino acid Tery-347, comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO. 3 or having at least 70%, preferably at least 80 %, most preferably at least 90 % sequence identity with the enzyme of SEQ ID NO. 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6, or

the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (MysB of HL69**),**

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 9 or the third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC of HL69**)**
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 60 or the sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO. 92 (O-methyltransferase from *Synechococcus* PCC 7335),
- a further recombinant gene encoding a further enzyme, wherein the further gene is selected from a group consisting of the following genes:

a) a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Tery_2971) or a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the sequence of SEQ ID NO: 15 (Ap3855),

b) a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 52 (Tery_2970), or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the sequence of SEQ ID NO: 12 (NpF5597) or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the sequence of SEQ ID NO: 74 (MysD_HL-69) or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the sequence of SEQ ID NO: 76 (Nv_MysD). In the case, that the further gene encodes a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 50, Tery-347.3 can be produced, which is a methylated mycosporine-2-glycine. In the case that the further gene is a fourth gene, encoding for the fourth enzyme as described above, a mixture of either Tery-347.2/Tery-347.1 can be produced, or in the case of using Nv_MysD as the fourth gene, Tery-347.1 can be formed. Tery-347.3 is a methylated mycosporine-2-glycine having an absorption maximum at 347 nm. Tery-347.2 is a methylated shinorine derivative also having its absorption

maximum at 347 nm. Tery-347.1 is a methylated porphyra-334 with an absorption maximum at 347 nm. The inventors of the present invention also have found, that homologous enzyme with a low degree of homology exist for the sixth enzyme being encoded by the sixth recombinant gene, namely Tery_2966. These enzymes can for example be O-methyltransferases from *Synechococcus* sp. PCC 7335 (49%) (SEQ ID NO: 92), *Chroococcidiopsis* sp. TS-821 (58%) (SEQ ID NO: 86), *Euhalothece* sp. KZN 001 (55%) (SEQ ID NO: 88), and *Chondrocystis* sp. NIES-4102 (50%) (SEQ ID NO: 90), wherein the percentages in parenthesis indicate the percentage of sequence identity to Tery_2966 as determined with the NCBI database search tool BLASTP 2.9.0+.

[0077] Subject matter of a further variant of the present invention is a mycosporine-like amino acid (Tery-347.1), being a methylated porphyra-334 and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 70,
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO. 72,
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 or the sixth recombinant gene encoding a sixth enzyme having at least 80% sequence identity to SEQ ID NO: 92 (O-methyltransferase from *Synechococcus* PCC 7335), and
- a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12. (NpF5597) or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably

at least 90% sequence identity with the enzyme of SEQ ID NO: 76 (Nv_MysD) or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 74 (MysD_HL-69)

[0078] Preferably, the mycosporine-like amino acid (Tery-347.1) exhibits a retention time on a HPLC system with an analytical ZIC-HILIC column (150X, 2.1 mm; 3.5 µm; 200 Å). In the area between 8.8 to 9.1 minutes, preferably 8.99 minutes. The inventors have found, that different Tery-347 MAA molecules can be produced, all of which show the maximum absorption of light at 347 nm, but which exhibit different chemical structures with different hydrophobicities and therefore show different retention times in a liquid hydrophobic interaction liquid chromatography (HILIC) (see also Examples 12 and 16). Mass spectrometry analysis of Tery-347.1 using a Sciex 5600 TripleTOF mass spectrometer revealed the molecular weight of the compound to be 360 Da. Tery-347.1 has the molecular formula $C_{15}H_{24}N_2O_8$.

[0079] Another embodiment of the present invention is directed to a mycosporine-like amino acid (Tery-347.2), being a methylated shinorine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70,
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72,
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 70%,

preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (O-methyltransferase from *Synechococcus* sp. PCC 7335), and

- a fourth gene encoding a fourth enzyme wherein the fourth gene is selected from a group consisting of:

a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 52 (Tery_2970), and/or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12 (NpF5597) or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 74 (MysD_HL-69). There is the possibility to employ three different fourth genes for the production of Tery-347.2, because a large variety of different fourth enzymes encoded by the fourth genes, for example Np_MysD (NpF5597) can attach serine to mycosporine-glycine resulting in Tery-347.2.

[0080] According to another variant of the mycosporine-like amino acid Tery-347.2, the retention time of this MAA on an HPLC system with an analytical ZIC-HILIC column (150X, 2.1 mm; 3.5 µm; 200 Å) is in the area between 10.1 to 10.3 minutes, preferably 10.26 minutes (see also Examples 12 and 16). Mass spectrometry analysis of Tery-347.2 using a Sciex 5600 TripleTOF mass spectrometer revealed the molecular weight of the compound to be 346 Da. Tery-347.2 has the molecular formula $C_{14}H_{22}N_2O_8$.

[0081] Another embodiment of the present invention is directed to a mycosporine-like amino acid (Tery-347.3), being a methylated mycosporine-2-glycine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68,
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70,

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72,
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (O-methyltransferase from *Synechococcus* sp. PCC 7335), and
- a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Tery_2971) or a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 (Ap3855).

[0082] A further variant of this mycosporine-like amino acid (Tery-347.3) exhibits a retention time on a HPLC system with an analytical ZIC-HILIC column (150 X 2.1 mm, 3.5 µm, 200 Ä) in the area between 10.0 to 10.2 minutes, preferably 10.1 minutes (see Examples 12 and 16). Tery-347.3 has the molecular formula $C_{13}H_{20}N_2O_7$. Its molecular weight as determined by using a Sciex 5600 TripleTOF mass spectrometer is 316. Based on the different retention times of the MAAs Tery-347.1, Tery-347.2 and Tery-347.3, it is clear that these compounds, although exhibiting the same absorption maximum at 347 nm, have different chemical structures with different hydrophobicities. As shown in Fig. 13B an E. *coli* strain #3186 expressing Ava_MysABC, Tery_2966 and a non-ribosomal peptide synthetase from *Calothrix* sp. NIES-2100 having a low sequence homology to the non-ribosomal peptide synthetase Tery_2968 can also produce a MAA compound with an absorption maximum at 347 nm (Tery-347.x). It is not known whether the Tery-347.x produced by this strain is one of the compounds Tery-347.1, Tery-347.2 or Tery-347.3 disclosed herein or a structurally distinct MAA also having an absorption maximum at 347 nm.

[0083] A further embodiment of the invention is directed to a mycosporine-like amino acid (Tery-347.4), being a methylated mycosporine-glycine-alanine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably

at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (MysA),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (mysB),
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC),
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (O-methyltransferase from *Synechococcus* sp. PCC 7335), and
- a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12 (NpF5597 or Np_MysD).

[0084] This novel MAA compound Tery-347.4 is a methylated mycosporine-glycine-alanine with the chemical formula $C_{14}H_{22}N_2O_7$. Its molecular weight is 330 Da as determined with a Sciex 5600 TripleTOF mass spectrometer.

[0085] Another embodiment of the present invention is directed to genetically modified cyanobacterial host cell for the production of a mycosporine-like amino acid Tery-322, comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having

at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant)

- a sixth recombinant gene encoding a sixth enzyme having at least 80% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase; low sequence homolog of Tery_2966). A search with the NCBI database (https://www.ncbi.nlm.nih.gov/) revealed that a couple of O-methyltransferases-like enzymes exist, which have a very low sequence homology with the O-methyltransferase Tery_2966 (sixth enzyme encoded by the sixth gene). In particular, these enzymes are *Synechococcus* sp. PCC 7335 O-methyltransferase (49%) (SEQ ID NO: 92), *Chroococcidiopsis* sp. TS-821 (58%) (SEQ ID NO: 86), *Euhalothece* sp. KZN 001 (55%) (SEQ ID NO: 88), and *Chondrocystis* sp. NIES-4102 (50%), wherein the percentages in parenthesis indicate the percentage of sequence identity to the enzyme Tery_2966 as determined with the NCBI database. Example 17 disclosed herein describes, that the O-methyltransferase-like enzymes from *Chroococcidiopsis* sp. TS-821, *Euhalothece* sp. KZN 001 (SEQ ID NO: 88) and *Synechococcus* sp. PCC 7335 catalyze the same reaction as Tery_2966 leading to the production of Tery-322.

[0086] Such a genetically modified cyanobacterial cell can furthermore comprise a eighth recombinant gene encoding an eighth enzyme for the production of Tery-364, the eighth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 56 (Tery_2968) or the eighth recombinant gene encoding an eighth enzyme having at least 80%, preferably at least 90% sequence identity to *Euhalothece* sp. KZN 001 (low sequence homolog of Tery_2968). Surprisingly, homologous proteins with a very low degree of homology to the enzyme Tery_2968, a non-ribosomal peptide synthetase, were found, which potentially catalyze the same reaction. In particular, these enzymes are the non-ribosomal peptide synthetases from *Oscillatoria* sp. PCC 10802 (53%) (SEQ ID NO: 94), *Chlorogloeopsis fritschii* (51%) (SEQ ID NO: 96), *Cyanothece* sp. PCC 7424 (49%) (SEQ ID NO: 98), *Nostocales cyanobacterium* HT-58-2 (49%) (SEQ ID NO. 100), *Scytonema cf. crispum* UCFS15 (49%) (SEQ ID NO: 102), and *Euhalothece* sp. KZN 001 (44%) (SEQ ID NO: 104) and *Calothrix* sp. NIES-

2100, (50 %). The percent numbers in parenthesis indicate the percentage of sequence identity to the protein Tery_2968.

[0087] Another embodiment of the present invention is directed to a genetically modified cyanobacterial host cell for the production of the mycosporine-like amino acid palythine, comprising:

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant)
- a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 or at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 (Tery_2971, MysD1 and Ap_MysD), and
- a seventh gene encoding a seventh enzyme having at least 80%, preferably at least 90% sequence identity with SEQ ID NO. 106 (*Calothrix* sp. NIES-2100, low sequence homolog of Tery_2972, clavaminic acid synthetase-like). Surprisingly, homologous enzymes for the seventh enzyme encoded by a seventh gene, Tery_2972 were found when searching the NCBI database, namely SEQ ID NO. 190 *Scytonema tolypothrichoides* VB-61278 (63%), and *Calothrix* sp. NIES-2100 (61%). The percent numbers in parenthesis indicate the percentage of sequence identity to the protein Tery_2972. Example 17 disclosed herein describes that *Calothrix* sp. NIES-2100 catalyzes the same chemical reaction as Tery_2972. The present invention therefore also discloses alternative enzymes, which can be used for either the sixth enzyme, the seventh enzyme or the eighth enzyme and which still catalyze the same chemical reaction.

[0088] The invention disclosed herein also describes genetically modified host cells, cyanobacterial cells, wherein each of the recombinant genes is under the transcriptional control of a promoter which is an inducible promoter, and which for example can be induced by the addition of an inducer or which is a constitutive promoter. The promoter can in particular be an inducible promoter which can be inducible by a change of a metal-ion concentration, further wherein the promoter is preferably inducible by an increase in the zinc concentration, such as the PsmtA or by an increase in the copper concentration, such as the PpetE promoter. Likewise, the mycosporine-like amino acids disclosed herein can all be produced by culturing the host cell, wherein any of the recombinant genes in the host cell are under the transcriptional control of the above described promoters.

[0089] According to another embodiment, the genetically modified host cell can be a host cell selected from the following group of host cells or the mycosporine-like amino acids disclosed herein can be produced by the cultivation of the following host cells selected from a group consisting of bacteria and eukaryotic cells, preferably actinobacteria and enterobacteria, such as *E. coli*, and more preferred cyanobacteria, such as *Cyanobacterium* sp., further wherein the cyanobacterial host cell is preferably *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4. In particular ABICyano1 and ABCyano4 can produce high amounts of mycosporine-like amino acids and are also easy to cultivate as described herein.

[0090] Both of these cyanobacterial species exhibit an extracellular capsular polysaccharide layer (CPS), wherein the CPS of ABCyano4 is particularly thick and can have a thickness of between 1 to 3 μm, preferably between 1.1 to 2 μm. These CPS layers are particularly advantageous, because a large fraction of the MAAs produced during the cultivation can stay associated with these CPS layers, so that the MAAs can be particularly easy isolated from a culture, by centrifuging the culture and further isolating the MAAs from the cells as described herein.

[0091] Preferably, anyone of the recombinant genes or all recombinant genes are from a cyanobacterium. This variant of the present invention and particularly refers to the first to ninth recombinant genes, coding for the respective enzymes for the production of the MAAs. Such a variant of the present invention is particularly preferred, when the host cell is a cyanobacterial host cell, such as ABICyano1 or ABCyano4.

[0092] Furthermore, anyone of the recombinant genes can be codon optimized for improved expression in the cyanobacterial cell, in order to enhance the number of copies of active enzymes in the cell, leading to a higher production rate of the mycosporine-like amino acids.

[0093] According to another embodiment of the present invention, anyone of the recombinant genes is located on a modified endogenous or a heterologous extrachromosomal plasmid.

[0094] Particularly preferred is, that any one of the recombinant genes is located on an endogenous extrachromosomal high copy plasmid, being present in a cell in at least 10, preferably at least 20, most preferably at least 40 copies per cell. As described herein, both of the preferred cyanobacterial strains, such as ABICyano1 and ABCyano4 include endogenous high copy plasmids, such as endogenous plasmids pABCyano4-B and pABCyano4-C in ABCyano4 or p6.8 in ABICyano1. Integrating the recombinant genes in an endogenous high copy plasmid, for example via homologous recombination ensures a high copy number of recombinant genes (high gene dosage) for MAA production in the host cells, thereby leading to a higher production rate of the MAAs.

[0095] According to another variant of the present invention, a method for producing an MAA in a cyanobacterial cell or in the host cell is disclosed, comprising:

a) growing any of the genetically modified cyanobacterial cells or host cells disclosed therein in the culture medium, and

b) isolating the MAA from the cells and/or the culture medium.

[0096] In particular, during step a) the host cells can be grown with light and CO_2 addition, if the genetically modified host cells are cyanobacterial cells, which are photoautotrophic.

[0097] During the method of producing the MAAs, at least 60%, more preferred at least 70% of the MAAs produced, is associated with the cyanobacterial cell and wherein in step b) the MAAs are isolated from the cells by separating the cells from the culture medium and isolating the MAAs from the cells. After separation of the cyanobacterial cells from the culture medium for example by centrifugation, the MAAs can be isolated from the cells as disclosed herein, in particular in example 11.

[0098] As disclosed herein, the cyanobacterial cell can have a capsular exopolysaccharide layer (CPS) wherein the MAAs produced are associated with the cell via the CPS.

[0099] Another embodiment of the present invention is directed to a pharmaceutical composition or a cosmetic composition comprising a UV absorbing compound, wherein the UV absorbing compound is an MAA that has been produced in the genetically modified cyanobacterial cell or host cell as described herein.

Definitions

[00100] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[00101] The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical value/range, it modifies that value/range by extending the boundaries above and below the numerical value(s) set forth. In general, the term "about" is used herein to modify a numerical value(s) above and below the stated value(s) by a variance of 20%.

[00102] The term "cyanobacterium" refers to a member from the group of photoautotrophic prokaryotic microorganisms which can utilize solar energy and fix carbon dioxide. Cyanobacteria are also referred to as blue-green algae.

[00103] The terms "host cell" and "recombinant host cell" are intended to include a cell suitable for metabolic manipulation, e.g., which can incorporate heterologous polynucleotide sequences, e.g., which can be transformed. The term is intended to include progeny of the cell originally transformed. In particular embodiments, the cell is a prokaryotic cell, e.g., a cyanobacterial cell. The term recombinant host cell is intended to include a cell that has already been selected or engineered to have certain desirable properties and to be suitable for further genetic enhancement.

[00104] "Competent to express" refers to a host cell that provides a sufficient cellular environment for expression of endogenous and/or exogenous polynucleotides.

[00105] As used herein, the term "genetically modified" refers to any change in the endogenous genome of a wild type cell or to the addition of non-endogenous genetic code to a wild type cell, e.g., the introduction of a heterologous gene. More specifically, such changes are made by the hand of man through the use of recombinant DNA technology or mutagenesis. The changes can involve protein coding sequences or non-protein coding sequences, including regulatory sequences such as promoters or enhancers.

[00106] The terms "polynucleotide" and "nucleic acid" also refer to a polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs

thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs. It will be understood that, where required by context, when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

[00107] The nucleic acids of this present invention may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages, charged linkages, alkylators, intercalators, pendent moieties, modified linkages, and chelators. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions.

[00108] The term "nucleic acid" (also referred to as polynucleotide) is also intended to include nucleic acid molecules having an open reading frame encoding a polypeptide, and can further include non-coding regulatory sequences and introns. In addition, the terms are intended to include one or more genes that map to a functional locus. In addition, the terms are intended to include a specific gene for a selected purpose. The gene can be endogenous to the host cell or can be recombinantly introduced into the host cell.

[00109] In one aspect the invention also provides nucleic acids which are at least 60%, 70%, 80% 90%, 95%, 99%, or 99.5% identical to the nucleic acids disclosed herein.

[00110] The percentage of identity of two nucleic acid sequences or two amino acid sequences can be determined using the algorithm of Thompson et al. (CLUSTALW, 1994, *Nucleic Acids Research* 22: 4673-4680). A nucleotide sequence or an amino acid sequence can also be used as a so-called "query sequence" to perform a search against public nucleic acid or protein sequence databases in order, for example, to identify further unknown homologous promoters, which can also be used in embodiments of this invention. In addition, any nucleic acid sequences or protein sequences disclosed in this patent application can also be used as a "query sequence" in order to identify yet unknown sequences in public databases, which can encode for example new enzymes, which could be useful in this invention. Such searches can be performed using the algorithm of Karlin and Altschul (1990, *Proceedings of the National Academy of Sciences U.S.A.* 87: 2,264 to 2,268), modified as in Karlin and Altschul (1993, *Proceedings of the National Academy of Sciences U.S.A.* 90: 5,873 to 5,877). Such an algorithm is incorporated in the NBLAST

and XBLAST programs of Altschul et al. (1990, *Journal of Molecular Biology* 215: 403 to 410). Suitable parameters for these database searches with these programs are, for example, a score of 100 and a word length of 12 for BLAST nucleotide searches as performed with the NBLAST program. BLAST protein searches are performed with the XBLAST program with a score of 50 and a word length of 3. Where gaps exist between two sequences, gapped BLAST is utilized as described in Altschul et al. (1997, *Nucleic Acids Research*, 25: 3,389 to 3,402).

[00111] Database entry numbers given in the following are for the CyanoBase, the genome database for cyanobacteria (http://bacteria.kazusa.or.jp/cyanobase/index.html); Nakamura et al. "CyanoBase, the genome database for *Synechocystis* sp. strain PCC6803: status for the year 2000", *Nucleic Acid Research*, 2000, Vol. 18, page 72.

[00112] The enzyme commission numbers (EC numbers) cited throughout this patent application are numbers which are a numerical classification scheme for enzymes based on the chemical reactions which are catalyzed by the enzymes.

[00113] "Recombinant" refers to polynucleotides synthesized or otherwise manipulated in vitro ("recombinant polynucleotides") and to methods of using recombinant polynucleotides to produce gene products encoded by those polynucleotides in cells or other biological systems. For example, a cloned polynucleotide may be inserted into a suitable expression vector, such as a bacterial plasmid, and the plasmid can be used to transform a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell" or a "recombinant bacterium" or a "recombinant cyanobacterium." The gene is then expressed in the recombinant host cell to produce, e.g., a "recombinant protein." A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

[00114] The term "homologous recombination" refers to the process of recombination between two nucleic acid molecules based on nucleic acid sequence similarity. The term embraces both reciprocal and nonreciprocal recombination (also referred to as gene conversion). In addition, the recombination can be the result of equivalent or non- equivalent cross-over events. Equivalent crossing over occurs between two equivalent sequences or chromosome regions, whereas nonequivalent crossing over occurs between identical (or substantially identical) segments of nonequivalent sequences or chromosome regions. Unequal crossing over typically results in gene duplications and deletions. For a description of the enzymes and mechanisms involved in

homologous recombination see Court et al., "Genetic engineering using homologous recombination," *Annual Review of Genetics* 36:361-388; 2002.

[00115] The term "non-homologous or random integration" refers to any process by which DNA is integrated into the genome that does not involve homologous recombination. It appears to be a random process in which incorporation can occur at any of a large number of genomic locations.

[00116] The term "expressed endogenously" refers to polynucleotides that are native to the host cell and are naturally expressed in the host cell.

[00117] The term "operably linked" refers to a functional relationship between two parts in which the activity of one part (e.g., the ability to regulate transcription) results in an action on the other part (e.g., transcription of the sequence). Thus, a polynucleotide is "operably linked to a promoter" when there is a functional linkage between a polynucleotide expression control sequence (such as a promoter or other transcription regulation sequences) and a second polynucleotide sequence (e.g., a native or a heterologous polynucleotide), where the expression control sequence directs transcription of the polynucleotide.

[00118] The term "vector" as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which generally refers to a circular double stranded DNA molecule into which additional DNA segments may be ligated, but also includes linear double-stranded molecules such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme.

[00119] Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply "expression vectors").

[00120] A "promoter" is an array of nucleic acid control sequences that direct transcription of an associated polynucleotide, which may be a heterologous or native polynucleotide. A promoter includes nucleic acid sequences near the start site of transcription, such as a polymerase binding site. The promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. The term

"promoter" is intended to include a polynucleotide segment that can transcriptionally control a gene of interest, e.g., a pyruvate decarboxylase gene that it does or does not transcriptionally control in nature. In one embodiment, the transcriptional control of a promoter results in an increase in expression of the gene of interest. In an embodiment, a promoter is placed 5' to the gene of interest. A heterologous promoter can be used to replace the natural promoter, or can be used in addition to the natural promoter. A promoter can be endogenous with regard to the host cell in which it is used or it can be a heterologous polynucleotide sequence introduced into the host cell, e.g., exogenous with regard to the host cell in which it is used. Promoters of the invention may also be inducible, meaning that certain exogenous stimuli (e.g., nutrient starvation, heat shock, mechanical stress, light exposure, etc.) will induce the promoter leading to the transcription of the gene.

[00121] The term "recombinant nucleic acid molecule" includes a nucleic acid molecule (e.g., a DNA molecule) that has been altered, modified or engineered such that it differs in nucleotide sequence from the native or natural nucleic acid molecule from which the recombinant nucleic acid molecule was derived (e.g., by addition, deletion or substitution of one or more nucleotides). The recombinant nucleic acid molecule (e.g., a recombinant DNA molecule) also includes an isolated nucleic acid molecule or gene of the present invention.

[00122] The term "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

[00123] The term "endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

[00124] The term "fragment" refers to a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence substantially identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in

length from at least about 6, 50, 100, 200, 500, 1,000, to about 1,500 or more consecutive nucleotides of a polynucleotide according to the invention.

[00125] The term "open reading frame," abbreviated as "ORF," refers to a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

[00126] The term "upstream" refers to a nucleotide sequence that is located 5' to reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[00127] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[00128] The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

[00129] As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence.

[00130] The term "substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript.

[00131] The terms "restriction endonuclease" and "restriction enzyme" refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

[00132] The term "expression", as used herein, refers to the transcription and stable accumulation mRNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

[00133] An "expression cassette" or "construct" refers to a series of polynucleotide elements that permit transcription of a gene in a host cell. Typically, the expression cassette includes a promoter and a heterologous or native polynucleotide sequence that is transcribed. Expression cassettes or constructs may also include, e.g., transcription termination signals, polyadenylation signals, and enhancer elements.

[00134] The term "codon" refers to a triplet of nucleotides coding for a single amino acid.

[00135] The term "codon–anticodon recognition" refers to the interaction between a codon on an mRNA molecule and the corresponding anticodon on a tRNA molecule.

[00136] The term "codon bias" refers to the fact that different organisms use different codon frequencies.

[00137] The term "codon optimization" refers to the modification of at least some of the codons present in a heterologous gene sequence from a triplet code that is not generally used in the host organism to a triplet code that is more common in the particular host organism. This can result in a higher expression level of the gene of interest.

[00138] The term "transformation" is used herein to mean the insertion of heterologous genetic material into the host cell. Typically, the genetic material is DNA on a plasmid vector, but other means can also be employed. General transformation methods and selectable markers for bacteria and cyanobacteria are known in the art (Wirth, Mol Gen Genet. 216:175-177 (1989); Koksharova, *Appl Microbiol Biotechnol* 58:123-137 (2002). Additionally, transformation methods and selectable markers for use in bacteria are well known (see, e.g., Sambrook et al, supra).

[00139] The term "selectable marker" means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, i.e., resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, spectinomycin, kanamycin, hygromycin, zeocin, chloramphenicol, and the like.

[00140] A "polypeptide" is a polymeric compound comprised of covalently linked amino acid residues. A "protein" is a polypeptide that performs a structural or functional role in a living cell.

[00141] A "heterologous protein" refers to a protein not naturally produced in the cell.

[00142] An "isolated polypeptide" or "isolated protein" is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids).

[00143] The term "fragment" of a polypeptide refers to a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide. Such fragments of a polypeptide according to the invention may have a length of at least about 2, 50, 100, 200, or 300 or more amino acids.

[00144] A "variant" of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements.

[00145] In one aspect the invention also provides polypeptides or proteins that have amino acid sequences that are at least about 50%, 60%, 70%, 80% 90%, 95%, 97%, 99%, 99.5% or more identical to the amino acid sequences disclosed herein.

[00146] The term "primer" is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

[00147] The term "polymerase chain reaction," also termed "PCR," refers to an in vitro method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under

quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[00148] The term "mycosporine-like amino acid" ("MAA") refers to a low molecular weight compound containing a 6 carbon ring structure, substituted with at least one amino acid moiety. Exemplary MAAs include, for example, mycosporine-glycine, shinorine, and mycosporine-2-glycine. These molecules absorb UV light, and exhibit photoprotection functions.

[00149] The term "UV" refers to light between the wavelengths of 280 – 400. This is divided between "UV-A" and "UV-B".

[00150] The term "UV-A" refers to light between the wavelengths of 315 – 400 nm.

[00151] The term "UV-B" refers to light between the wavelengths of 280 – 315 nm.

[00152] The term "selection-free" refers to a growth medium that does not include a selection agent, such as an antibiotic, that would allow only cells having a functional selectable marker gene to survive.

[00153] The term "plurality" means more than one.

[00154] In the following, the term "heterologous gene" in the context of genes coding for enzymes involved in the production of MAA and the term "MAA gene" will be used interchangeably. If more than one heterologous gene coding for enzymes involved in the production of MAA is present in a genetically modified cyanobacterial cell, then these genes will be referred to as the first heterologous gene, the second heterologous gene and so on.

[00155] In the following the terms "extracellular polysaccharides" (EPS) and "capsular polysaccharides" (CPS) will be used interchangeably. These terms refer to the extracellular polysaccharide layer of the cyanobacterial host cells of the present invention, in particular the extracellular polysaccharide layers of *Cyanobacterium* sp. host cells, such as *Cyanobacterium sp.* ABICyano1 and *Cyanobacterium* sp. ABCyano4.

Genes and Gene Clusters for the Production of MAAs

[00156] A large variety of different enzymes is involved in the synthesis of mycosporine-like amino acids (MAAs). Fig. 1 shows the general pathways for synthesizing MAAs, also naming enzymes, which were not yet implicated in the synthesis of MAAs. Starting with the compound sedoheptulose-7-phosphate, MysA, a family of homologous enzymes, which among others include the homologous enzymes Ava_3858, MysA_HL-69, and Tery_2977 convert this compound into

desmethyl-4-deoxygadusol. This intermediate compound is then subsequently converted into 4-deoxygadusol by the enzyme family MysB, which among others include the homologous enzymes Ava_3857, MysB_HL-69, and Tery_2976. Subsequently, glycine can be attached to 4-deoxygadusol resulting in Mycosporine-glycine catalyzed by the enzyme family called MysC, which among others include the enzymes Ava_3856, MysC_HL-69, and Tery_2975. Starting with mycosporine-glycine, different amino acids can be attached to this molecule by different enzyme families all named MysD. Glycine can be attached to mycosporine-glycine resulting in mycosporine-2-glycine catalyzed by either one of the enzymes Ap3855 (Ap_MysD) or Tery_2971. The attachment of alanine to mycosporine-glycine leads to mycosporine-glycine-alanine catalyzed by the enzyme Np_MysD (NpF5597 of *Nostoc punctiforme).* Threonine can be attached to mycosporine-glycine via the catalytic action of any of the enzymes MysD_HL-69, Np_MysD (D-ala-D-ala ligase NpF5597 from [*Nostoc punctiforme*]) or Nv_MysD (D-alanine-D-alanine ligase MysD [*Nostoc verrucosum* KU005]) resulting in porphyra-334. Finally, serine can be attached to mycosporine-glycine catalyzed by the enzymes MysD_HL-69, Tery_2970, or Np_MysD resulting in shinorine.

[00157] Fig. 39 depicts further proposed pathways for the formation of mycosporine-like amino acids, which are partly known from the literature. Some of the MAAs, such as Tery-322, are a methylated mycosporine-glycine, Tery-364, Tery-347.3, which is a methylated mycosporine-2-glycine, and Tery-347.2 which is a methylated shinorine are not yet known from the literature.

[00158] The following Table 1A includes a description of the genes used to genetically modify the cyanobacterial host cells for MAA production and their respective SEQ ID Nos in the sequence listing:

Table 1A: Recombinant Genes and enzymes involved in MAA production encoded by the recombinant genes

Description	SEQ ID NO:
Recombinant gene encoding 3-dehydroquinate synthase from *Anabaena variabilis* (Ava_3858) GenBank: ABA23463.1	1
Ava_3858(ABICyano1opt) codon optimized variant of Ava 3858 for ABICyano1	2
Enzyme for MAA production encoded by the gene Ava_3858(ABICyano1opt)	3

Description	SEQ ID NO:
Recombinant gene encoding O-methyltransferase (Ava_3857) from *Anabaena variabilis* GenBank: ABA23462.1	4
Ava_3857(ABICyano1opt) codon-optimized variant of Ava_3857 for ABICyano1	5
Enzyme for MAA production encoded by the gene Ava_3857(ABICyano1opt)	6
Recombinant gene encoding ATP-grasp enzyme-like protein from *Anabaena variabilis* (Ava_3856) GenBank: ABA23461.1	7
Ava_3856(ABICYANO1opt) codon-optimized variant of Ava_3856 for ABICyano1	8
Enzyme for MAA production encoded by the gene Ava_3856(ABICyano1opt)	9
Recombinant gene encoding D-ala-D-ala ligase (NpF5597) from *Nostoc punctiforme* ATCC 29133GenBank: ACC83902.1	10
NpF5597(ABICyano1opt) codon-optimized variant of NpF5597 for ABICyano1	11
Enzyme for MAA production encoded by the gene NpF5597(ABICyano1opt)	12
Recombinant gene encoding D-ala-D-ala ligase (Ap3855) from *Aphanothece halophytica* GenBank: BAO51916.1	13
Ap3855(ABICyano1opt) codon-optimized variant of Ap3855 for ABICyano1	14
Enzyme for MAA production encoded by the gene Ap3855(ABICyano1opt)	15
Recombinant gene encoding Amino acid adenylation nonribosomal peptide synthetase (NRPS) (Ava_3855) GenBank: ABA23460.1	16
Ava_3855(ABICyano1opt) codon-optimized variant of Ava_3855 for ABICyano1	17
Enzyme for MAA production encoded by the gene Ava_3855(ABICyano1opt)	18
Recombinant gene encoding MysAB 3-dehydroquinate synthase O-methyltransferase fusion protein *Porphyra umbilicalis* GenBank: OSX70340.1	19
mysAB (ABICyano1opt) *Porphyra umbilicalis* codon-optimized variant of mysAB for ABICyano1	20
Enzyme for MAA production encoded by the gene mysAB (ABICyano1opt) *Porphyra umbilicalis*	21
Recombinant gene encoding MysCD ATP-grasp enzyme-like protein D-ala-D-ala ligase fusion protein *Porphyra umbilicalis* GenBank: OSX70339.1	22

Description	SEQ ID NO:
mysCD (ABICyano1opt) *Porphyra umbilicalis*, codon-optimized variant of mysCD for ABICyano1	23
Enzyme for MAA production encoded by the gene mysCD (ABICyano1opt) *Porphyra umbilicalis*	24
codon-optimized variant of mysCD ATP-grasp enzyme-like protein D-ala-D-ala ligase fusion protein *Chondrus crispus* GIDCcT00003179001	25
Enzyme for MAA production encoded by the gene mysCD (ABICyano1opt) *Chondrus crispus*	26
Recombinant gene encoding dehydroquinate synthase MylA [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54016.1	27
Enzyme for MAA production encoded by the gene dehydroquinate synthase MylA [*Cylindrospermum stagnale* PCC 7417]	28
Recombinant gene encoding O-methyl transferase MylB [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54017.1	29
Enzyme for MAA production encoded by the gene MylB [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54017.1	30
Recombinant gene encoding D-alanine-D-alanine ligase MylC [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54018.1	31
Enzyme for MAA production encoded by the gene MylC [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54018.1	32
Recombinant gene encoding ATP-grasp domain protein MylD *[Cylindrospermum stagnale* PCC 7417] GenBank: ANS54019.1	33
Enzyme for MAA production encoded by the gene MylD [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54019.1	34
Recombinant gene encoding ATP-grasp domain protein MylE [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54020.1	35
Enzyme for MAA production encoded by the gene MylE [*Cylindrospermum stagnale* PCC 7417] GenBank: ANS54020.1	36
Recombinant gene encoding desmethyl-4-deoxygadusol synthase MysA [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12742.1	37
Enzyme for MAA production encoded by the gene MysA [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12742.1	38
Recombinant gene encoding O-methyltransferase MysB [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12743.1	39
Enzyme for MAA production encoded by the gene MysB [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12743.1	40
Recombinant gene encoding D-Ala D-Ala ligase MysD [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12744.1	41
Enzyme for MAA production encoded by the gene MysD [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12744.1	42

Description	SEQ ID NO:
Recombinant gene encoding ATP-grasp ligase MysC2 [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12745.1	43
Enzyme for MAA production encoded by the gene MysC2 [*Nostoc commune* var. flagelliforme QSY 1] GenBank: AQU12745.1	44
Recombinant gene encoding ATP-grasp ligase MysC1 [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12746.1	45
Enzyme for MAA production encoded by the gene MysC1 [*Nostoc commune* var. *flagelliforme* QSY 1] GenBank: AQU12746.1	46
Recombinant gene encoding Putative Clavaminic acid synthetase–like Tery_2972 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52130.1	47
Enzyme for MAA production encoded by the gene Tery_2972 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52130.1	48
Recombinant gene encoding D-alanine-D-alanine ligase 1 Tery_2971 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52129.1	49
Enzyme for MAA production encoded by the gene Tery_2971 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52129.1	50
Recombinant gene encoding D-alanine-D-alanine ligase 2 Tery_2970 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52128.1	51
Enzyme for MAA production encoded by the gene Tery_2970 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52128.1	52
Recombinant gene encoding 6-phosphogluconate dehydrogenase, NAD-binding Tery_2969 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52127.1	53
Enzyme for MAA production encoded by the gene Tery_2969 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52127.1	54
Recombinant gene encoding Amino acid adenylation domain Tery_2968 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52126.1	55
Enzyme for MAA production encoded by the gene Tery_2968 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52126.1	56
Recombinant gene encoding conserved hypothetical protein Tery_2967 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52125.1	57
Enzyme for MAA production encoded by the gene Tery_2967 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52125.1	58
Recombinant gene encoding Hydroxyneurosporene-O-methyltransferase Tery_2966 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52124.1	59
Enzyme for MAA production encoded by the gene Tery_2966 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52124.1	60

Description	SEQ ID NO:
Recombinant gene encoding 3-dehydroquinate synthase Tery_2977 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52135.1	61
Enzyme for MAA production encoded by the gene Tery_2977 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52135.1	62
Recombinant gene encoding O-methyltransferase Tery_2976 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52134.1	63
Enzyme for MAA production encoded by the gene Tery_2976 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52134.1	64
Recombinant gene encoding ATP-grasp ligase Tery_2975 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52133.1	65
Enzyme for MAA production encoded by the gene Tery_2975 [*Trichodesmium erythraeum* IMS101] GenBank: ABG52133.1	66
Recombinant gene encoding 3-dehydroquinate synthase MysA [*Cyanobacterium stanieri* HL-69] GenBank: AUC61936.1	67
Enzyme for MAA production encoded by the gene mysA [*Cyanobacterium stanieri* HL-69] GenBank: AUC61936.1	68
Recombinant gene encoding O-methyltransferase MysB [*Cyanobacterium stanieri* HL-69] GenBank: AUC61935.1	69
Enzyme for MAA production encoded by the gene mysB [*Cyanobacterium stanieri* HL-69] GenBank: AUC61935.1	70
Recombinant gene encoding ATP-grasp ligase MysC [*Cyanobacterium stanieri* HL-69] GenBank: AUC61933.1	71
Enzyme for MAA production encoded by the gene mysC [*Cyanobacterium stanieri* HL-69] GenBank: AUC61933.1	72
Recombinant gene encoding D-alanine-D-alanine ligase mysD [*Cyanobacterium stanieri* HL-69] GenBank: AUC61934.1	73
Enzyme for MAA production encoded by the gene mysD [*Cyanobacterium stanieri* HL-69] GenBank: AUC61934.1	74
Recombinant gene encoding D-alanine-D-alanine ligase mysD [*Nostoc verrucosum* KU005] GenBank: BBC27544.1	75
Enzyme for MAA production encoded by the gene mysD [*Nostoc verrucosum* KU005] GenBank: BBC27544.1	76
Recombinant gene encoding D-alanine-D-alanine ligase [*Actinosynnema mirum* DSM 43827] GenBank: ACU38111.1	77
Enzyme for D-alanine--D-alanine ligase [*Actinosynnema mirum* DSM 43827] GenBank: ACU38111.1	78
Recombinant gene for Phosphopantethiene-protein transferase Tery_4684 [*Trichodesmium erythraeum* IMS101] GenBank: ABG53648.1	79
Enzyme for Phosphopantethiene-protein transferase [*Trichodesmium erythraeum* IMS101] GenBank: ABG53648.1	80

Description	SEQ ID NO:
Synthetic DNA sequence linkerX	81
Protein sequence linkerX	82
Synthetic DNA sequence linkerY	83
Protein sequence linkerY	84
Gene for MAA production hydroxyneurosporene methyltransferase [*Chroococcidiopsis* sp. TS-821] GenBank: PPS45853.1	85
Enzyme for MAA production hydroxyneurosporene methyltransferase [*Chroococcidiopsis* sp. TS-821] GenBank: PPS45853.1	86
Gene for hydroxyneurosporene methyltransferase [*Euhalothece* sp. KZN 001] GenBank: PNW38180.1	87
Enzyme for hydroxyneurosporene methyltransferase [*Euhalothece* sp. KZN 001] GenBank: PNW38180.1	88
Gene for hydroxyneurosporene-O-methyltransferase [*Chondrocystis* sp. NIES-4102] GenBank: BAZ46464.1	89
Enzyme for hydroxyneurosporene-O-methyltransferase [*Chondrocystis* sp. NIES-4102] GenBank: BAZ46464.1	90
Gene O-methyltransferase, putative [*Synechococcus* sp. PCC 7335] GenBank: EDX84454.1	91
Enzyme O-methyltransferase, putative [*Synechococcus* sp. PCC 7335] GenBank: EDX84454.1	92
Gene non-ribosomal peptide synthetase [*Oscillatoria* sp. PCC 10802] NCBI Reference Sequence: WP_017716158.1	93
Enzyme non-ribosomal peptide synthetase [*Oscillatoria* sp. PCC 10802] NCBI Reference Sequence: WP_017716158.1	94
Gene hypothetical protein PCC6912_44890 [*Chlorogloeopsis fritschii* PCC 6912] GenBank: RUR75917.1	95
Enzyme hypothetical protein PCC6912_44890 [*Chlorogloeopsis fritschii* PCC 6912] GenBank: RUR75917.1	96
Gene amino acid adenylation domain protein [*Cyanothece* sp. PCC 7424] GenBank: ACK73338.1	97
Enzyme amino acid adenylation domain protein [*Cyanothece* sp. PCC 7424] GenBank: ACK73338.1	98
Gene non-ribosomal peptide synthetase [Nostocales cyanobacterium HT-58-2] GenBank: ARV60046.1	99
Enzyme non-ribosomal peptide synthetase [Nostocales cyanobacterium HT-58-2] GenBank: ARV60046.1	100
Gene non-ribosomal synthetase protein [*Scytonema cf. crispum* UCFS15] GenBank: ANY58991.1	101
Enzyme non-ribosomal synthetase protein [*Scytonema cf. crispum* UCFS15] GenBank: ANY58991.1	102

Description	SEQ ID NO:
Gene hypothetical protein BEN50_01875 [*Euhalothece* sp. KZN 001] GenBank: PNW38181.1	103
Enzyme hypothetical protein BEN50_01875 [*Euhalothece* sp. KZN 001] GenBank: PNW38181.1	104
Gene of hypothetical protein NIES2100_44520 [*Calothrix* sp. NIES-2100] GenBank: BAY24657.1	105
Enzyme of hypothetical protein NIES2100_44520 [*Calothrix* sp. NIES-2100] GenBank: BAY24657.1	106
recombinant gene for Phosphopantethiene-protein transferase Tery_4684 [*Trichodesmium erythraeum* IMS101] GenBank: ABG53648.1	179
Enzyme for Phosphopantethiene-protein transferase [*Trichodesmium erythraeum* IMS101] GenBank: ABG53648.1	180
recombinant gene encoding D-alanine-D-alanine ligase MysD [*Nostoc verrucosum* KU005] GenBank: BBC27544.1	181
Enzyme for MAA production encoded by the gene mysD [*Nostoc verrucosum* KU005] GenBank: BBC27544.1	182
recombinant gene for hypothetical protein (clavaminic acid synthetase) SD80_01665 [*Scytonema tolypothrichoides* VB-61278]GenBank: KIJ85249.1	189
hypothetical protein SD80_01665 (clavaminic acid synthetase) [*Scytonema tolypothrichoides* VB-61278]GenBank: KIJ85249.1	190
Recombinant gene for amino acid adenylation domain protein [*Calothrix sp. NIES-2100*] GenBank: BAY24658.1	192
amino acid adenylation domain protein [*Calothrix sp. NIES-2100*] GenBank: BAY24658.1	193
recombinant gene for 4'-phosphopantetheinyl transferase superfamily protein [*Calothrix sp. NIES-2100*] GenBank: BAY20437.1	194
4'-phosphopantetheinyl transferase superfamily protein [*Calothrix sp. NIES-2100*] GenBank: BAY20437.1	195

[00159] Table 1B includes a nomenclature for the recombinant genes and the respective enzymes encoded by these genes as used throughout this invention. Table 1B also lists the substrates of the various enzymes and the products into which the substrates are converted by the various enzymes. Some of the enzymes can recognize more than one substrate or are able to convert the same substrate into different products. For example the genes and respective enzymes of the MysD2 family, referred to as the fourth genes encoding fourth enzymes can convert mycosporine-glycine into either one of shinorine, porphyra-334, mycosporine-glycine-alanine:

Table 1B: Nomenclature of Genes and enzymes involved in MAA production

Nomenclature for gene/protein	Enzyme family	Substrate(s)	Product (s)	Examples
First	MysA	Sedoheptulose -7-phosphate	Desmethyl-4-deoxygadusol	Ava_3858, mysA_HL-69, Tery_2977
Second	MysB	Desmethyl-4-deoxygadusol	4-deoxygadusol	Ava_3857, mysB_HL-69, Tery_2976
Third	MysC	4-deoxygadusol	Mycosporine-glycine	Ava_3856, mysC_HL-69, Tery_2975
Fourth	MysD2 (Shi/P-334)	Mycosporine-glycine	Shinorine, porphyra-334, mycosporine-glycine-alanine	Tery-2970, NpF5597, mysD_HL-69, Av_mysD, Amir_mysD
Fifth	MysD1 (M-2-Gly)	Mycosporine-glycine	Mycosporine-2-glycine	Tery_2971, Ap3855
Sixth	O-methyltransferase	Shinorine/porphyra-	Tery-347.1, Tery-347.2, Tery-347.3	Tery_2966, OMT_PCC7335, OMT_

		334/mycosporine-2-alanine		TS821, OMT_KZN001, …
Seventh	Clavaminic acid synthetase-like	Mycosporine-2-glycine	palythine	Tery_2972, CAS_NIES2100, CAS_VB-61278
Eighth	Nonribosomal peptide synthetase	Tery-322	Tery-364	Tery_2968, NRPS_KZN_001, NRPS_NIES2100, Ava_3855, …
Ninth	Hydroxy-isobutyrate dehydrogenase-like	shinorine	Mycosporine-methylamine-serine, asterina-330	Tery_2969, HIBD_CS-953, HIBD_PCC8106, HIBD_PNG, HIBD_PCC7420

[00160] Methods to modify cyanobacterial cells with genetic modifications to produce compounds of interest are also described in U.S. Patent No. 9,315,820, U.S. Patent No. 9,965,364, U.S. Patent No. 9,551,014, U.S. Patent No. 9,476,067, U.S. Patent No. 9,493,794, and U.S. Patent No. 9,353,400, all of which are incorporated by reference herein in their entireties.

Codon Improvement of Recombinant Genes

[00161] At least some of the recombinant MAA genes to be expressed in cyanobacterial host cells can be codon improved for optimal expression in the target 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* 325:728–730; 1987). Codon

improvement (sometimes referred to as codon optimization or codon adaptation) can be performed to increase the expression level of foreign genes. The codon usage for ABIcyano1 is the same as for ABCyano4. Therefore, we refer to Table 3, the codon usage table of ABICyano1, which is disclosed in the PCT application WO 2014/100799A2 and which is incorporated in its entirety.

Choice of Promoters

[00162] The inserted genes can be controlled by one promoter, or they can be controlled by different individual promoters. The promoters can be constitutive or regulatable. The promoters can be, for example, inducible. The promoter sequences can be derived, for example, from the host cell, from another organism, or can be synthetically derived.

[00163] Any desired promoter can be used to regulate the expression of the inserted MAA biosynthesis genes. Exemplary promoter types include but are not limited to, for example, constitutive promoters, regulatable promoters such as inducible promoters (e.g., by nutrient source, nutrient starvation, heat shock, mechanical stress, environmental stress, metal concentration, specific metabolites, light exposure, etc.), endogenous promoters, heterologous promoters, and the like. Suitable promoter sequences are also disclosed, for example, in U.S. Patent No. 9,315, 820, U.S. Patent No. 9,551,014, PCT/EP2012/067534, U.S. Patent No. 9,476,067, U.S. Patent No. 9,157,101, PCT/US2013/077364, U.S. Patent No. 9,493,794, and PCT/US2015/000210, all of which are hereby incorporated by reference in their entireties.

[00164] The recombinant MAA biosynthesis gene(s) can be under the transcriptional control of a constitutive promoter. In this way, a sustained level of transcription and, therefore, enzymatic activity of the corresponding protein can be maintained during the whole period of cultivation. For example, the constitutive promoter can be endogenous to the cyanobacterial cell. This has the advantage that no recombinant transcription factor has to be present in the host cell. The endogenous promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

[00165] Suitable constitutive promoters include, without limitation, the PrpsL promoter (Gene ID: ABICyano1_orf1758), PpsaA promoter (ABICyano1_orf3243), PpsbB (ABICyano1_orf2107), PcpcB promoter (ABICyano1_orf2472), PatpG (ABICyano1_orf1814), PrbcL promoter (ABICyano1_orf1369), and variations thereof. Further suitable endogenous constitutive promoters from genes with unknown function exhibiting appropriate transcriptional activity include, without limitation, the promoters of Gene IDs ABICyano_orf1924,

ABICyano_orf1997, ABICyano_orf3446, ABICyano_orf0865, ABICyano_orf1919, ABICyano_orf3278, ABICyano_orf1181, ABICyano_orf1627, ABICyano_orf0265 and ABICyano_orf2536, ABICyano_orf0615, and variants thereof.

[00166] In an embodiment, the promoters can be derived from the cyanobacterial strain *Cyanobacterium* sp. PTA-13311, or they can be derived from another cyanobacterium or from another organism. In an embodiment, the promoters can be about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100 % identical to the promoter sequences described herein.

[00167] The promoters can be regulatable promoters, such as inducible promoters. For example, certain promoters are up-regulated by the presence of a compound, which is called the inducer, while other promoters can be up-regulated by the absence of a compound (also termed "repressible").

[00168] Various promoters that can be used include promoters that are regulatable by the presence (or in other promoters, by the absence) of inductors such as different metal ions, different nutrient sources, different metabolites, different external stimuli such as heat, cold, salinity or light. In some embodiments, the regulatable or inducible promoters are induced under conditions such as nutrient starvation or nutrient source, stationary growth phase, heat shock, cold shock, oxidative stress, salt stress, light, darkness, metal ions, organic chemical compounds, and combinations thereof. For example, a particularly tight control of the expression of gene can be achieved if a gene is under the transcriptional control of a Zn-, Ni-, Cu-, or Co-inducible promoter. Exemplary Zn-regulatable promoters and their variants are described, for example, in International Application No. PCT/EP2013/077496. Exemplary Zn, Ni, and Co-regulatable promoters are described, for example, in International Application No. PCT/2012/076790, both of which are incorporated by reference herein in their entireties.

[00169] In a further embodiment, the regulatable or inducible promoter is inducible by a change of a metal-ion concentration. Such a change of metal-ion concentration includes for instance the addition or depletion of certain metal ions. Suitable inducible promoters include, without limitation, the PziaA promoter, the PsmtA promoter, PaztA promoter, the PcorT promoter, the PnrsB promoter, the PpetJ promoter, the PpetE promoter, the Porf0316, the Porf01460 promoter, the Porf0221 promoter, the Porf0223 promoter, the Porf3126 promoter, the PmntC promoter, and variations thereof. In particular, the promoter being inducible by a change of a metal-ion concentration can be inducible by Zinc-ions and/or Cu-ions, such as the PsmtA promoter or the PpetE promoter.

[00170] Preferably, the regulatable or inducible promoter is endogenous to the cyanobacterial cell. An endogenous inducible promoter is usually well-recognized by the metabolically enhanced cyanobacterial cell without the need to introduce further genetic modifications.

[00171] In further embodiments, the choice of regulatable or inducible promoters can include, but are not limited to, PntcA, PnblA, PisiA, PpetJ, PpetE, PggpS, PpsbA2, PsigB, PlrtA, PhtpG, PnirA, PnarB, PnrtA, PhspA, PclpB1, PhliB, PcrhC, PziaA, PsmtA, PcorT, PnrsB, PnrsB916, PaztA, PbmtA, Pbxa1, PzntA, PczrB, PnmtA, PpstS, and the like.

[00172] The inducible promoter can, for instance, also be a nitrate inducible promoter. Suitable nitrate inducible promoters include, without limitation, the PnirA promoter, the PnrtA promoter, the PnarB promoter, and variations thereof.

[00173] In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence, 5'-untranslated region and/or the ribosomal binding site (RBS) can be used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction. In some preferred variants, the different inducible promoters are inducible by different metal ions.

[00174] The promoters PhspA, PclpBl, and PhliB can be induced by heat shock (raising the growth temperature of the host cell culture from 30°C to 40°C), cold shock (such as, for example, reducing the growth temperature of the cell culture from 30°C to 20°C), oxidative stress (for example by adding oxidants such as hydrogen peroxide to the culture), or osmotic stress (for example by increasing the salinity). The promoter PsigB can be induced by stationary growth, heat shock, and osmotic stress. The promoters PntcA and PnblA can be induced by decreasing the concentration of nitrogen in the growth medium and the promoters PpsaA and PpsbA2 can be induced by low light or high light conditions. The promoter PhtpG can be induced by osmotic stress and heat shock. The promoter PcrhC can be induced by cold shock. An increase in copper concentration can be used in order to induce the promoter PpetE, whereas the promoter PpetJ is induced by decreasing the copper concentration. Additional details of these promoters can be found, for example, in PCT/EP2009/060526, which is incorporated by reference herein in its entirety.

[00175] In an embodiment, the promoters of any of the above embodiments may be selected from the endogenous inducible promoters identified in *Cyanobacterium* sp. with the ATCC accession number PTA-13311 ("ABICyano1") as listed in table **2A** below, and variants thereof.

Table 2A: *Cyanobacterium* sp. ABICyano1 endogenous promoter sequences

Promoter	Sequence	SEQ ID NO:
PnirA	AATTAATAACTTCTTCCTGTACGGGCGAATGGCCA TTTGCTCCTAACTAACTCCGTACTGCTTTGCGGAAC GAGCGTAGCGAACTCTCCGAATTACTAAGCCTTCA TCCCTGATAGATGCAAAAAACGAATTAAAATTATG TGTAAAAAGAAAATGTGTCTTTATTTAGTAGTCAA AGTTACAAAATATTAAGAATCAAATTAATAATGTA TTGGGCAGTTAAGTATATAAGTCTTTAAATATTTAT TTGTATTCAATATATTAACCGAGGACAAATT	107
Porf3126 (PsmtA)	CCAATATCTTGTCATACATACTTATTTGCCTCACTA TTAGCCCTATATGTCTCTATTGTATTTTTCTTTTTCT CCTATTCCTAGATCTTGTAATGAATCATTACTCTCT GAAATATAGCTACTAATTTTATGGTTGTTTGTAAA ATATATTAACAAATGAACAATAAATCATATTTTGT GTTAATCTAATTATTAGACAACTACTGAATTTATAT TCAGATATTCACAGATAGGAGAATTTTGATT	108
PnrtA	TATTATTTTTCGTTTATATGCAGATTTAGAATAAAC AAAATTCATTTACTGCAAATTTTCAAAAAAATGTG ACTAAACATACAAAATAAAGAAAAAATAAAGTTT TAAATTTATGTACATCAAACTTAAGAAATGTTTAA ATTACTTAGAAATTTATAGTTC	109
Porf3461 (petJ)	TTTATATATAAACTCGAATAAAATTATCAATATAA AGTCAAACTATATCTATCCTATTTTAACTGCTATTG GTAAGTCCCTTAATTAGTGTTGGGGTGAATAGATT TTAAAAGGGCAAACCCCCCTTTATCCTCCCTCGAG AGGGGGGAGGGCAAAAGGCAAGGGGCAAGGGAA AAATTAAGAATTAAGAATTAAAAACTCCGAACACC TGTAGGGGCGAATAGCCATTCGCTTCCCCTCATCC CCCCATCTCCCCAACACCCTAAGCCCCTACTCGTTA CTCATTTATTTACATCATTTATTTACATCATTAAGA AAAGTAACAAATTTTGACAAGTAGTCTTTTGACAG GAAAAAGCAAATTCTCGAAGATGAAAACAATAGA AAAAAATTCAATCTTACAGTAACGATGAAAAAACT TTTAGGCTTAATT	110
PnarB	TGTCTCAAAAAGACAGGTTTTTTTTATGAAAGTAA TAAGAAATAAGTAGAAGTGAGGAGTTGGAAAGAT AGGATTAAGAATTAGGAGTTAACTATTTTCATTCTT TATTCTTCCATTGCCCATTGAGAAATCATATCTAAA ATCAGCAACGCCAAATTTTAGATGCAAAATAACCA TAAATAAAATGCAGAAAAAAGAATACTTTAGATCT TCCGTATCAGAAGATACATTTCTTAACAAAATCTG GTGACAAGATTAAACACACGAAATCCGAGGTTTTA TATATTGATTAGTCCTAG	111
Porf1071 (PmntC)	ATTCTGTGAATTGATTAGATTTGAGGTTTTTTAAGA GGTTGATTACCTTGCCTCCAAAAAAATCATAACAC	112

	ACTAATGCTCTATATGAAAGGGCTTTAGACCCATA GGTTTTTGAGAAAAAAACTTGCTAACTCTCGGACA ATGTCAGCATAACTAAAGTCAATTCTTTTCGTACTT TATAATTGTCTATAATTTAATATACAACTGTTCTGA AACTAGTTTTTCTCTACATTCCTTAGTTTTATCTGA GTAAGGTTGCTTGTAACTTAACTTCGGTTGGGCCT AAAAATATCCGATTAGGAGCAGGTGTCAGACTTTA ATTAATTATTAATTATTAATTGCTTATTGCCAACCC TCGGCGACACCACTTTTTCATCAGCCCCAGATAAA GATTGATGTTTTAGTTTTGTTTCTTTTTATCCCCTAA TTCAACTAATACAAGTAAAACTAAGGTTGTTTATC AAAAATGATGGTTGATGTTTGGGTAAATTTTAAGA TATTATGAAAAGAAAATGAATAAAAAATGAAAAA TCTTT	
Porf0221	GAATATCTCATCCTTAGCTTCTACTTATACCTTCAG CATAGTTAAAAATCATCCCTTTATTGATGGTAATA AAAGAACAGGTTTTATTAGTGGAGTAACCTTTTTA ATGCTCAATGGTTCTCACTTTACTGCTTCTGAAGTG GAAGTAGTACATATCATCCAAACCTTAGCTAGTGG CAGAATTACCGAGGAAGAATTACAACAATGGTTCG TAAGGAAAAGTAAGCAGATGAATAATTAAAGCAT CATTTCATCCTCATTTCATATTCTCCTGTCACCATG GTATGGAAGATTAGGTAAAAATGAGGAAAAAGTT TATT	113
Porf0223	ATACATGGTTGGTTCACTGACTTTTACCCCAGTTTT CTCTTTGAACAATTGGCATAACTCTGAAAAAATCA GATCGGGCTTTTGTTGAATTATTTGTTCAATCAAAG CAAAACCGTGATTGTCTATTTTCTTTTTTTTCCCAC CACTCATAGATAAAAATTTATCCCGAACTCAGGTT ATATTAAGTTCGGATGATCACTTAAGATAATTGAT CAGATTGGTTAAGATAGAGAAAAATTCTTTTTCAT AGTGATTTCATAATTGATAGTTACAATAACGATTA TTATTTAGTAAAAAGATTTTCAAATC	114
Porf0316	TGGTCAAGTTACTATATGTTTAGAAACAACAAAAA AAGAAGTCATTATAAAAATAATTGATACAGGAATT GGCATTAATAAAGAAGAACAAAAATTAATTTTTAA TCGTTTTTATCGAATCAATAAAGCAAGAAATAGAG AGAAAGGCAGTTGCGGATTAGGTTTAGCTATTGCA AATGCGATCGCGCTTAATCATGGTGGTAGAATAAT TTTAGAAAGTCAAGAAAATCAAGGCAGTATTTTTA CCGTTTATTTACCGAAAATCATTTCATCCTAATTTC ATATTCTTTTGACAGAATCAAAGGTAAAGATAAAA AGAGAGAAACAGTC	115
Porf0128	CCTCAACTACAAGTTCTTTTATATATTACTTTAACC TGAGTTTTGGATAAGCTGAAAGCATTATTTTCTCGT AGTCAGAAAACCTTATAGCTTCTTAGAAATAACGA TAAAATTACCTTAATCCGAACTGACGTTAAATATA TTCACCCCTATCACCCCAAAACCCTAAGCCCCTAC TTCCCCCTTTCCCTTCATCACCTCATCCCCCCATCC CCTAACACTTAACCTTATTCTTTATTCTTAAACCGA ACTGAGGTGAAGTTGCAGAATACCCATGGGGGGTT ACAGCATTGTAGAAAAATAAATATTCTTTCATTAT	116

	TAAGGTTGTTTGGTAAAAATATGTGAAAACCCTAA TAATT	
Porf1486	GGGGACAGACATATTTTTATCATAATGGTAAATTC ATAATAATTTTAGACTTTTTTTTTGCAAAAATTAATC TCACTCTCTTCTTTCCCTATCTCCCATTGTTTCTTAT ATCCCAATGCCCCAATACCCAAAGCTCAGAAAATA GGTATTAGCGAAGAGGTGTTGATCCCCTCCCCTAG CAAAATATACTCCTATATAGTAAAGTGAGAAAGTG AAGAAATAAGATCAAGTTCGCAATTT	117
Porf3293	TTGACGATTGTATTGACTTACGCCAAATGGCTTAC CCTCATAGTGAATAGTTGATAATTAAGAATTAAAA ATCCCGTTCACGACAGAAGGGAGTGTAAGAGCCTT CGGTGCGAACTCTCATCTTCCCTGAAACCTGACAC CTGAAACCTGACACCTGAAACCTGACACCTCATCT CCCTAATCCCCTAATTTTAATGAAAAAATACCCTG AGTGGGCATTGAAAAAAAAGAAAAGTTGTTCGAC TATGAAATAAGAATTCTGCACTTCGTGAGAAAAAA GGAAATGAAAT	118
Porf3621	CTATTTAACTAGGAAAAGGTAAAGTTAAAAGGAC AAGGGTAAATAATTAAAAATTAAGAATTAAGAAC TTCTAACTCTCATTACTCATTACTTATTTCCTCCTCT CACCCCTTCTCCTGATCACCTCTTCTCCTCAATACT CGGAACTCATTTCCCCATGGTGTGACACTCAAATC AAAAGTCTGTTATTGACTTTCAGATGAAATATTAC TATGATAACAATATCCCCCCTATGGGTATATAAAA ATATGAGCGATATTAGTTAAAAATCAAATTTGGAT TTTTTTTCTGAAAATATTTTAAGATTAAGTAAAGAT AAGTAAAGAAATTATAAGCAATTTTGTTAAATCAT ACC	119
Porf3635	CTCACACTGAAAATATTGCCACAAGAAATAAAGAT CAAGCAATAATCCTGACTAAAAAGGAATAAAGTA ATTATCCTTTTCCTGATATGTTATCTGACTTGTTGTT TCTTAGTCATGTTCCTTCCATTTTTATTTTTGTTTTT ATCATTTTTATTACAAAAATTTCTTAATAGGGCTAA AGCATTTAGTTAGTTTTTTAGCTCTCAACAAGTTGA CTAATCAATATAATGCCCTAAGTTAATTTGCCCTTG GTTTGACGGAGGATATTGGAAAAAAGAAACTTCTC GTTGTATTTCACAGGGAAAAGGGGGAAATTTTATT AATAACTAAACAATAGAAAATAATTATTTATTTAT ATTATTTTGTGAACAAATGTTCAAGAATTAAAGTG TAATAAGAAAATTTATTTTTTTATATTTATTTAAAA CTTAGATATAAGCCTAAAGGTCTGAAATTATTATT AGACAATCAATTGATTCAGAGGTAATAGTTTTTTA CTTAAAAATATTTTTTCAAAATTATCCCCTATTTGG GTATTGAAAAATAAATAAATTCAAGTAATAATATA CAGAATAAAGGAAAATCTAATCTTAAAAATTTTGT GTGTGAGGAATTGAAA	120
Porf3164	CAAATCACGAGAATTTATGTAGGGACTATTTTGGG TTGACGGTGGAGAGTATGTCGCCCTTGAATTATGA CCCGAAGATGAAGATGTCGGGGAGGTGGAAGGAC GGTCTTTAAGAGGTTTAACATCAAAGTTGGTCATA ATCTCTGTCCCTGTTTGATAACTACTATTTAATTTT	121

	GAGTTGTTTTAGGTACATCAAAATACCCAAATCCT TACTCTCCCCTCAATATACAACAAAAAAAACTTTT TGATTCACTTTAGTCATAAAAATTAGAATTTATCTA CCGAAATATTACATAAATGTAATGTATATATTTTCT GATTTATTCCGTGTGAGCCATGATTCATAATTTATA ATTCATAATTTCTAAATATGCCCCTACAATGGATAT AGAATGTCATTTTAATTATAGGTATCATAATCGTG GTAGTTACTCCGGAAAAAACTATTGAATCAAATTC AGTCTCACCTGCTACAGATAGAGTAGCCGTTATTC TT	
Porf1072	CTACAGGGGCAAGATTTGGCGGAAATCTATATGTG GATTCTCTTTCAAGTGAAGAAGGTGCAGTGCCGAC TTATCTGGACTTATTAGAATACGATATTCGCACTAT TACTAATGGTTTGTTAGCAGGAGTGAACAATTAAA AATTTTTTCCTAATTGACGAATAAAAAATCAATGT CAACTAATAGTTAACAATACTCTCTGAAAACCAAA AATTGTCAACCAAAACATAACATAATTTTTACCCA AAAACCTCATTTATAAACTTTAAGGATAAAATCAA TG	122
Porf1074	GGGATTAGAGAGTTCAAAGTTAGGAATGAGGTGTC AGGTTTTAGGTTTCAGGTTTAGGGGAGCAATGAGA AAGAGGTTTCAGGTTTCAGGTGTCAGGTTGCAGGT GTCACAGGTGATGAGGGGATGGGGGATGAGGGGG AAACAAGTAAGTAATAAGTGTTCGGAGTTTTTAAT TCTTAATTCTTAATTTTTCCTTTGCCTCTTGCCTTTT GCCTTGTCTTAATTACTAATTTCTAATTAAAATGAT TGTGTTTTCTAGTTTAGTCTCATGGTTACTTGAACC CTTACAGCATAGTTTT	123
Porf01075	TTACAAACGGCGGGAATTATTATGGTAGTAGCGAT GTTAGTAACCCCGGGTGCGATCGCATATTTACTTA CAGATCGTTTTGATCAAATGTTAATCTTATCAATAG TTAGTAGTGTTCTATCTTGTGTTTTAGGCACTTATT TAAGTTATCATTTTGATGTTTCTACGGGGGGAAGT ATTGTCGTTTTAATGACCATAATTTTTATTTTAGCG ATGATTTTTGCTCCTAAATATGGCATCATCAATCAA AATACCAAAATATATTCTGCTTAACTTGTTTACTGA TACTTCAAATAATCATATAACCTATCTTCCGAGTTA AAAATAATGGATATTATCCAACTGAGGTCGAGAAT AGAGTTTCTTTTTTGATAGAATTTTTTTACACCAGT TATTCATTACTATCATGGGATAAT	124
Porf1542	TAATATAGTGATTATTATAAATGCAATGTGAATCA AACCTATATTTTACCGTACATTGACCATGGAACTT AATTTGAGGTGATTAGTAGAGGGTGCGATCGCCCT ATTTGTCAAATAATAAAGATAACATTTGACATTGC TGATTGAAGACATAAAACACAGAAAAAATCAGGT AAAAATATAAAGCTAAAGTCTAAATATGGTTTACT TTTGCCTTCGACTTACAACAAAAAATCATAGCTAG AATCACCAACGCCTAATATTTTATTTAGCTGAAATT TTGGGATGAACTTTTTGTAAAAATCGGGGGTCTAA AAATATAGCAACCACGATATTAAATAACTGAGTGA TTATTTTAATCTATTGGGGGCTTATTAACTAAATAC TTGCATTTTTATGGAGGGTTTTAATT	125

Porf1823	AAAGATTATTTTCTACAGAAGCAACCCTTTCATCTT CCGAATTTTCAGGAATTTCCTGCTTTTGTTTCTGAA TATTAGCATAGGCGGCTTTTGCCCACTCTAAAGAA GGTTGAGACTGAATTTCTGAGGTTTCAGAAGGAGC ATTAGATTGTTTATCTTCAACAACAGGAGGTTTTTG TTCAATATTTTCCTTATTCTCTTTTTTACGGCGAAA CCAATTAAACATAATGATTGTGCATAAATATTCGT TAATATATTGTAACCCTAGAAAGGAATCGGTTTCA GGTTTATCCCCAGAGAATGTGAACCTTTACAGAAA GTAAAAAGTCTAAAATCGTAGCAACAATAAATCAC AGAAATTGAG	126
Porf0222	GCGATTATCAACCACGAAAACATACAATTATTATC AAACCTGCTGAGAAATTATCCACAGAAATAGATGT TTCTGCGAAGGGAAAATGGGCTTTTCATTGCCATT TAATGTATCACATGGATGTGGGAATGTTTCGGACT ATTAATGTTATTTCCTAAAAAATAATAGTATTAAA GCCTAAAATTTTTATAAAAAAATTCATGTCTTTTAT TAGGGTGAGCATTCTTCCTTTATGTCTCCTTATTTT ACCTCTTTAGAGGTAACTACAAACTTAATCAAAAA ATTTAGATAATTAATTATATCA	127
Porf3232	CATCTTTACTTTTGACTAACATTTCATAGGTATCAT GACGAAAATTTTTTAGTCTGTTATATTTGTTCATGT AGAGAGATTTTAATTTGTGATTATTTTATTTTCTCT CTATTTTTCTTTTTTGTCTTGTCCTTCCTCATTTTTCT CTACATTTAGTCTAAACTACAGCTCTTTAATCTTCA GTTTCTCTTTCCTCCTCTTCCTCATCAAGGTAATCA TCCCAATTAATATCTTCTTCTTGTTCTAATTTGGGT TGAGATTGTTGTTTATCAATCATATTTCATACTCCT AAAACTTTCTTACTTATTTATCAGTTACTTTTTACC CATTTATGCAATAGTGTAGAAATTTTTTTCGATCGA GTTAATTAATTTTTATTTCAACCATATCTAAATAAT TCTTGATGGACATTCTAGTTAACTAGAAGGTTTAA GCTAAAAATAATTATTGATATTGCCTTCGGTATAA CTAACTATATCCAGAGAAAAAG	128
Porf3749	CTCAAGAGATAGTTAAAAAACAAATAGCTTTAGTC TATCAATTAATCGAATTATTTTTACAAACAAATTTT CATAAACCCATAGAACTAGAGGAGGAAGTTATTTA TGTTTAAAAATCTAAAAGAGTTTTATATTCCCCTAA AACCCCCTTAGTAAGAGTGACTTTTTTCATCATTTG CCTGTAAATTCTCCTCTTTTAATAAGAGAGCTAGG GTGTTTTAAAAGAGGATTTTATTGCTTTCCAATTCT AACTACTTCAAAAACTTATTTTATACTCAATAATTT ATTAATCAAGAGGAAATTACC	129
PrbcL	TCGAGCGCTCGTTCCGCAAAGCGGTACGGAGTTAG TTAGGGGCTAATGGGCATTCTCCCGTACAGGAAAG AGTTAGAAGTTATTAATTATCAACAATTCTCCTTTG CCTAGTGCATCGTTACCTTTTTAATTAAAACATAAG GAAAACTAATAATCGTAATAATTTAACCTCAAAGT GTAAAGAAATGTGAAATTCTGACTTTTATAACGTT AAAGAGGGAAAAATTAGCAGTTTAAAATACCTAG AGAATAGTCTGGGGTAAGCATAGAGAATTAGATTA GTTAAGTTAATCAAATTCAGAAAAAATAATAATCG TAAATAGTTAATCTGGGTGTATAGAAAATGATCCC	130

	CTTCATGATAAGATTTAAACTCGAAAAGCAAAAGC CAAAAAACTAACTTCCATTAAAAGAAGTTGTTACA TATAACGCTATAAAGAAAATTTATATATTTGGAGG ATACCAAC	
PrnpA	AATAGTTGATAATTACTCGTTACTCATTACTCACTT AAACCTGCCACCTGATACCTGCCACCTCTCCCCCC ATCACCTCATCCCCTCAACATTCCGAACCCCTTGAC ACTTTGAACTAAAATTGTATTAAAGTGCAAATCTG GACGGGGTTAACCAGTGTGACTTATAATAGTAAAC GCTGTTTTTTATAATAAATAAGCTAAATATTTAAA AACTATGAGTAAATATACACTAAATGGTACTAGAC GTAAGCAGAAAAGAACCTCCGGTTTCCGCGCCCGT ATGAGAACCAAAAATGGTAGAAAAGTAATTCAAG CTCGTCGTAATAAGGGTAGAAAAAGATTAGCAGTA TAAAATTACTGTTAAATAAGGAAGCTAAGTTTAGC ATTTTAAGTTTGATATTACTAATCATTAAATTTACT GTGAAATATAGGTGGGACTACCATCAAAGCATCGA CTGAAACGGCGTTTAAATTTCCAATCTGTTTATCAA CAGGGTATTCGCCGCTCTAGTCGTTATTTTATTGTC CGAGGGTTACGG	131
PrpsL	CTCCGCTTAAAAAATTTCATTTTTCGATCAAAAAA GACAAATTATTACTAATTAGCTCATGGCAATAAAT AATCAGTAGTAATCTGTTTTCACATTTTATTGTTAA TTTTTATTATTGCTAATATCAACCTTTTCTACTTCTG CTTAATATTTTATTTATGCTCAATGGGAAAATCTGA AATAAGATTGAGAACAGTGTTACCAATAGAAGTAT TTAAGGTTTAAAGCATACCTTAAAGATAACATTTT TTTTTGAAAAGAGTCAAATTATTTTTGAAAGGCTG ATATTTTTGATATTTACTAATATTTTATTTATTTCTT TTTCCCTTAAAATAAGAGCTAAATCTGTTTTTATTA TCATTTATCAAGCTCTATTAATACCTCAACTTTTTC AAGAAAAAATAATAATAATTTTTCCCTCTATTCTC ATGACCTTTTAGGAAAATTAATTTTAGAAAAACTA TTGACAAACCCATAAAAAAATGAGATAAGATTATAG ATTGTCACTGGTATTTTATACTAGAGGCAAATTAT ATTTATATATACAAAAATGCTGTATAAAAAACATC T	132
PrpoA	AGTAAAGATTATCACCAACATCTGAAACCTGACTT CATCAACTGAGGAAATAACCACTGTGGCTGTGTTT AAAATCGACTGCGTAGCAAGTAAAACTCAAAAAA ATCAAGGTCAATACGGAAAGTTTGTGCTTGAACCC TTAGAAAAAGGACAAGGCATAACT	133
PpsaA	CTACATCAACTAATCAAAAGTTAAGAAAAAAGAT AGAAACGCCCATGAATATTAAAGATTAATCTGTGT CCTTTAACTTTTTATCCCCTTAAAAGAGCATAACTA AAACATTGATAGATTTTATAAAGAAAAGTAACAAA ATCTTGACTTAAATGAGAAAGGATTAAAAACCAAA GCCTTATCTGAGGGAATGTTAAACAAATTTTAAAT ATTGTTAAGCAAGAACCACAATGGTGACAAATAGC CCTTATCATCTTCAGTAATGTAGTAGTTTAAGTATT TGTCGAGAGAGGAATCCCTC	134

PpsbA2	GATCGAATTTTTGACTATTTAATAATTTCTTTACTA TTCATAATATCTCAAAAGACTTCTATCTTTTTAAGT AAACTACCTCCTCTAAGAATAAACACTTATTGACT ATATTCCTTTTTAGTTATAAAATGGCATTTAAAGTT ACTCAAAATATTTGCAATCATTCTACAAAACATAG TGTATTTCCTTGTATTAAGCGTATTGTGTCCTGTTA GATAATGTAGGAAAGATTGTGAGTTGATAGGTGAT AAATACATAACTCATTAGACAACAAGATAAAGTTG TAGGAGTTCTAAATT	135
PpsbD	AAGAGTTTGGCATTTTTATTGGTAAGACTATTCTGA GAAAAATGTGACAATTTGTTAAAATATTTGCTAGA AATAGAAAAAGTAATTTGGCAAAGATACTTAAATC GTATCGAAAAACGGAGTTACATTAACTCTAACTCA TGCTATATTAAGAAAAGTTAATTGCAGATCAGTAT TATTGCTGAGTAGCAGTGCCGTCTCCAATAATATA AAGAGAGACAATATAAAAGTAAAACTTGACAAGT TAAAAAAAGAAAGATT	136
PcpcB	AACTTTAGATATTCGTAGTTGGCAATGTCGTAAAT GCGGAACAATACATGGAAAACATATAGATTTGTAA TGAGAAAAAGTGTAAACAAATATTAAGAAAAAGA TCAGAAAAATTTAACAACACGTAATAAAAAAATG CGTCACTACGGGTTATAAATTTACATGAAAGGTTA AAACACTTTTCTGAGACGATTTTGATAAAAAAGTT GTCAAAAAATTAAGTTTCTTTACAAATGCTTAACA AAAACTTGGTTTTAAGCACAAAATAAGAGAGACTA ATTTGCAGAAGTTTTACAAGGAAATCTTGAAGAAA AAGATCTAAGTAAAACGACTCTGTTTAACCAAAAT TTAACAAATTTAACAAAACAAACTAAATCTATTAG GAGATTAACTACA	137
PcpcB*3	AACTTTAGATATTCGTAGTTGGCAATGTCGTAAAT GCGGAACAATACATGGAAAACATATAGATTTGTAA TGAGAAAAAGTGTAAACAAATATTAAGAAAAAGA TCAGAAAAATTTAACAACACGTAATAAAAAAATG CGTCACTACGGGTTATAAATTTACATGAAAGGTTA AAACACTTTTCTGAGACGATTTTGATAAAAAAGTT GTCAAAAAATTAAGTTTCTTTACAAATGCTTAACA AAAACTTGGTTTTAAGCACAACGCAAGAGAGACTA ATTTGCAGAAGTTTTACAAGGAAATCTTGAAGAAA AAGATCTAAGTAAAACGACTCTGTTTAACCAAAAT TTAACAAATTTAACAAAACAAACTAAATCTATTAG GAGATTAACTACA	138

[00176] In an embodiment, the promoters of any of the above embodiments may be selected from the endogenous inducible promoters identified in *Cyanobacterium* sp. with the ATCC accession number PTA-125253 ("ABCyano4") as listed in table **2B** below, and variants thereof.

Table 2B: *Cyanobacterium* sp. ABCyano4 endogenous promoter sequences

Promoter	Sequence	SEQ ID NO:
PnirA	CATTCCCTCATCTCTCTCCCCAATAGATGCAAAAAACGAATT AAAATTATGTGCAAAAAGAAAATAAGCCTTTATTTAGTAGT CAAAGTTACAAAATGTTAAGAATCAAATTAATAATGTATTG GGCAGTTAAGTATATAAATCTTTAAATATTTATTTGTATTCA ATATATTAATCGAGGACACATT	139
PsmtA	CTCCTATTCCTAGATCTCGTAATGAATCATTACTCTCTGAAA TATACCTCAATTTTATGGTTGTTTGTAAAATATCTTAATAAA TGAACAAGCAATCATATTTTGTGTTAGTCTAATTATTAAATA ATTACTGAATCTATATTCAGATATTCACAAATAGGAGAATTT TGATT	140
PpetE	AAATTAAGCAAAAAACTCTCTTCACAGAAGACAATGAAAAC CTTATTTACGTATTACTGAGACCCGAATCTTACGCCCACCGT AAGCATCCCGTATTGCTGCTACCTTCCGGTCCTGACAAGGTT TGAGCGTCACAGTTGCATAAGTCCGAGTCAGTGACCATTAT AACTTAATCTCCTCAACAAACACAAGGTTAATTGTCCAAAA CAATTTTTTAAATTAGATATGACATTAGGTCAAAGTTCATAG TATGATAGTAGAAAATAAAATTTGACGATCTGTAAAAATAA AAAAAATACA	141
Porf01460	TTAATTTCGTAACAAATAATACTTAATTTTTAACCCTTTATTT TATCCCAAACTCAAATCTATTCTTAAATCTTAATTACCCTTT ACCCAAACCTAAACAGTAAACCTGTAAACTAGCTTAAAAGT AATTGACTCTCTAGCCTACTAGAAGGTTTAACGTATAAATA GAACAATTTTTTCCTTTAGTGAATTGGAATTGAACTGATT	142
PrbcL	CGATGCTAGAGATGAATTTATATATCAAACAGACTTAGTGA GAAGGGCAAAAAGATATTTTCACCGTGATAATTTACCTTAT CCTTTACTTCCCCCCTTAATCATAATTAGGGCTGTTTGGGGA AATTTCTTTTTTATCTGAACTCCGAACTCAAATAATTGTCAT TTTCTTCATGATATAAGAAAAACTAATAACTATAATAATAT AATCTCAAAGTGTAAAGAAATGTGAAATTTTGACCTTTATA CCGTTAAAAAAAGCAAAATGAGCAGATTAAAATACCCAGA GAATAGTCTAGGGTAAGCATAGAGAATTAGATTAGTTAAGT TAATCAAATTCAGAAAAAATAATAATCGTAAATAGTTGATC TAGGTGTATAGAAAATGATCCCCTTCATGATAAGATTTAAA CTCGAAAAGCAAAAGCCAAAAAACTAACTTCCATTAAAAG AAGTTGTTACATATAACGCTATAAAGAAAATTTATATATTTG GAGGATACCAAGC	143
PpsaL	TACGCCTCGCTTTCGATTTGTAAGAATAATAATCCCATTACG ACAGCAGGCATAACTAAGCCAACAGCAGGAACTAAAATGG CAGGTAAGAAAGAAGCCGCAAAATCTCCAAGAATCATTTTT TTTATTTTCCTATACTAAAAAAGTTAACCTAGATGGATATTA CTGCTAAGTTTAACCAAAAAGAATCAAATATAAAGATTCTT AACAAAAAAAATGTGAATAGACTCCGATCAAGTTAAAGTAA GCTAAAAATACTTAATTATTTGATAACCAATTTAAGGAGAT CGTATAAAAAACA	144
PpsaA	TCGATTGTTAAGGGTTAATTGTCAATTACTAAATGTAAAAG AAAAAATTTATTATTAATATTTGTTTTTGTCTATAGTCAGTTT GATAAATTATGATATCTACTTGAGTCAATCAAAAGTTAAGA AAAAACAGAGAAACTCCCATGAACATTAAAGATTCATCTCC ATCTTTTTATTTTTTATCCCCTTAAAAGACCATAACTAAAAC ATTGATAGACTTTATAAAGAAAAGTAACAAAATCTTGACTT AAATGAGAAAGGATTAAAAACCAAAGCCTTATCTGAGGGA ATGTTAAATAAATTTTAAATATTGTTAAGCAAGAACCACAA	145

	TGGTGACAAATAGCCCTTATCATCTTCAGTAATGTAGTAGTT TAAGTATTTGTCGAGAGAGGAATCCCTC	
PpsaD	GAGGCGATCGCATCTTATTCTCAAGGATTTAAAGGGGCATA CCTATTCCAAAAACCAAACACAGACGAGGGAATAGCCTTTA TTCTTTGGGAAAAAATAGAAGATATGGAAGAAAACAAAAA CGAAGTCCATAACCAAATTATGGAACAAATGAAGCATTTAT TTGCTACCCCTCCTCAAACCAGTTTTTATGATGTTTTAACAG AATTTTCTCCTTAATAAGAATTTACACTTGAAGGATTAAAGA AATAAACTGTTACAGTTCATAACAAAGATGCACAATAATGG GGATCTCAGTGGTAGCATTGCCTAGATAGTAAATATAATTT GTACAAAAGCAGTATCTCT	146
PpsaF	TTAATCATTTCCAAATGACGACGGGAAGCCAACTCAGGCAC AACTCCGCCATACTGCTCATGTAGTTTGATTTGCGAAGCCAC TACATTAGCGATAACATTACGATTTGTTACAATGGCGACGC TAGTTTCATCACAACTACTTTCAACTGCTAAAATTGTGGGCA TTATTTTCTTGATTTGTTAAGAATAATAAATTTTCTAACTATT ACTTTACTCCATTTACTAATCACAGTAAAATCGCATTTGAAG TCATTTTTTCGGCTTCATTTTTTGTAACAAAAACTTATTGTTT CGTAGCAAAAGGAAACAATTTT	147
PcpcB	ATGAGAAAAAATGTAAACAAATATTAAGGAAAAGATCAGG AAAATTTAACAACACGTAATAAAAAAAGGTGTCACTACGGG TTATAAATTTACATGAAAGGTTAAAACACTTTTCTTAGACGA TTTTTGTAAAAAAGTTGTCAAAAAATTAAGTTTCTTTACAAA TGCTTAACAAAAACTTGGTTTTAAGCACAAAATAAGAGAGA CTAATTTGCAGAAGTTTTACAAGGGAATCTTGAAGAAAAAG ATCTAAGTAAAACTACTCTGTTTAACCAAAATTTAACAAATT TAACAAAACAAACTAAATCTATTAGGAGATTAACTAAAA	148
PpetE*	AAATTAAGCAAAAAACTCTCTTCACAGAAGACAATGA AAACCTTATTTACGTATTACTGAGACCCGAATCTTACG CCCACCGTAAGCATCCCGTATTGCTGCTACCTTCCGGT CCTGACAAGGTTTGAGCGTCACAGTTGCATAAGTCCG AGTCAGTGACCATTATAACTTAATCTCCTCAACAAACA CAAGGTTAATTGTCCAAAACAATTTTTTAAATTAGATA TGACATTAGGTCAAAGTTCATAGTATGATAGTAGAAA ATAAAATTTGACGATCTGTAAAAGGAGAATTTTGATT	149
Porf01460	TTAATTTCGTAACAAATAATACTTAATTTTTAACCCTTTATTT TATCCCAAACTCAAATCTATTCTTAAATCTTAATTACCCTTT ACCCAAACCTAAACAGTAAACCTGTAAACTAGCTTAAAAGT AATTGACTCTCTAGCCTACTAGAAGGTTTAACGTATAAATA GAACAATTTTTTCCTTTAGTGAATTGGAATTGAACTGATT	150
Porf01460*2	TTAATTTCGTAACAAATAATACTTAATTTTTAACCCTTTATTT TATCCCAAACTCAAATCTATTCTTAAATCTTAATTACCCTTT ACCCAAACCTAAACAGTAAACCTGTAAACTAGCTTAAAAGT AATTGACACTCTAGCCTACTAGAAGTGTTATACTATAAATA GAACAATTTTTTCCTTTAGTGAATTGGAATTGAACTGATT	151
PoprB	GCCCTAATAATTTTATAAAACAAGACAAAGTTAAACCAGTC TTAACCTTTAAGGTTTATGATGGACGATGAAAAAACACACC ATTATTTTCCAGCTACTTAATAGTTTTTTTTCCTATTTACTAT ATAATAGCTATCTGATAACAAAAAAATAAAAAAGTTGTTAT CTTAGTTACA	152
PrpsO	GGATTCGGAGACTACCCGCCATATTTCTACTGTTAGGGGAG TTGGCTACAAGTTTGATATTGAAAATAGTTAACCTAATTGTT GTAATTTATTTGTTTATGATAGCAGATTTGGAAAGAGAGAA TAGTTAATAGTTGATTATTAATAATATTATTCCGAACTCAAA TAGTTCCTAATTAAAATCAGTTTATGATATAATCATAGGT	153

PpsaC	GGAGTTTTCTTAATCTTAAGAGACACTGCAAATCTCGCTTTC TTCCAACAATTATTACCGGAATAGATTTTTACGGCACATTCA AAAGGTAATAGGTATAGGGTATCAGGTAAACCTAAAATGTA TTCAAATTGTATTTTTTGTTGCTTATTAATTTTTAAACTCAAT CATTGATTTTTATCTATCGTTGTCCATCCTAAAAGAAGAAAA ATTGTTACAGAGTTTGAAGTTATATATCAGAAAAGGTGACA GAGATTATATACTGCGTTTA	154
PcpcA	TATCCTCAGCGATTTCGGCAACAACTTTGGGATCGATGCCGT AGCCTAAGTTTCCCATTAAGGCTTCACCGCTTAATTTTAATA AAATCCGTTGGTAAGTCATTCCTCGGTTGCCTTCATATTCGA TAAATTTTCTTCAGTTAAGATACCAGTTTTTGACTACCATAA ATTAGTTTTTTTTATTAACAACTTACGATATGGATAATCAAT ATCCTCATCCCAATAATTGATAGGTTTTAATTTTCATTGACA AAACGCAATAAAACTTTACTAAATTCTAGGGAAAACTGCAC GGAGTTTGCACAAAGATTCTGTTTAAATAATCA	155

[00177] In certain other preferred embodiments, truncated or partially truncated versions of these promoters including only a small portion of the native promoters upstream of the transcription start point, such as the region ranging from -35 to the transcription start can often be used. Furthermore, introducing nucleotide changes into the promoter sequence, e.g. into the TATA box, the operator sequence and/or the ribosomal binding site (RBS) can be used to tailor or optimize the promoter strength and/or its induction conditions, e.g. the concentration of inductor required for induction.

[00178] The following Table 2C shows the nucleotide sequences of two intergenic regions IScpcBA and IScpcBA*1 employed to enhance the expression of the enzymes involved in the production of MAAs as described herein.

Table 2C:

Intergenic sequence	Sequence	SEQ ID NO:
IScpcBA	TTTAAACCAAGATTAGAAAATCCATTTCATTAACGT AAACCAACATAATTAGGAGAAATTAATTACA	156
IScpcBA*1	TTTAAACCAAGATTAGAAAATCCATTTCATTAACGT AAACCAACATAATTAGGAGGATCAGCACA	157

[00179] In an embodiment of the invention, the cyanobacterial host cell is *Cyanobacterium* sp. ATCC accession number PTA-13311 ("ABICyano1", also termed "AB1"). This strain has been found to grow well under various indoor and outdoor conditions, and can be genetically modified to produce a compound of interest. The strain, as well as its endogenous plasmid p6.8, has been described, for example, in U.S. Patent NO: 8,846,369, U.S. 9,315,832, and U.S. Patent NO: 9,157,101, all of which are hereby incorporated by reference in their entireties.

[00180] In another embodiment of the invention, the cyanobacterial host cell is *Cyanobacterium* sp. ATCC accession number PTA-125253, also called "ABCyano4" or also termed "ABCC1710". Similar to AB1, this strain has been found to grow well under various indoor and outdoor conditions, and can be genetically modified to produce a compound of interest. As it is shown herein, ABCyano4 can produce more biomass than AB1 in batch cultivation and exhibits a much thicker capsular exopolysaccharide layer (CPS layer) compared to AB1, so that MAAs produced with genetically modified ABCyano4 cells are mainly associated with this CPS layer or are produced intracellularly, enabling an easier purification of the MAAs. The strain, as well as its endogenous plasmids pABCyano4-B and pABCyano4-C (FIG. 40 and 41), has been applied for recombinant MAA production by insertion of MAA biosynthesis genes of interest from different cyanobacterial origin.

[00181] A deposit of the Algenol Biotech LLC's proprietary strain of Cyanobacterium sp., strain ABCyano4, disclosed above and recited in the appended claims has been made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The ATCC Accession Number is PTA-125253.

[00182] The 16S rDNA of *Cyanobacterium* sp. ABCyano4 shows a high sequence identity of around **99%** to the 16S rDNA sequences of different cyanobacterial species of the genus *Cyanobacterium*, including *Cyanobacterium* IHB-410, *Cyanobacterium aponinum* ETS-03, and *Cyanobacterium* sp. MBIC10216. The 16S ribosomal RNA (rRNA) gene sequences (16S rDNA) of ABICyanol was predicted from the genome sequence with the RNAmmer program (Lagesen K, *et al.* (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research* 35(9):3100-3108.), These sequences were then used as a query to search against the NCBl database and 16S rDNA sequences from 4 species belonging to the genus *Cyanobacterium* were retrieved as the top BLAST (Altschul SF, *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids. Res.* 25(17)3389-3402) hits. ABICyanol and ABCyano4 16S rDNA sequences are **99%** identical to the rDNA sequence of *Cyanobacterium* sp. ETS-03.

[00183] Integration of recombinant genes into endogenous plasmids of ABCyano4 was conducted via homologous recombination, a method well known to those skilled in the art. The integrative plasmids harbor an oriVT for conjugative transfer and homologous 2-4 kb flanking regions upstream and downstream of an integrative sequence followed by a resistance marker gene. In order to insert recombinant MAA synthesis genes into the endogenous plasmids

pABCyano4B and pABCyano4C respective integrative regions were applied as shown in Fig. 40 and Fig. 41.

[00184] In an embodiment, a culture of the cyanobacterial cell is grown in an outdoor photobioreactor system, and the MAA that is produced can be isolated, for example, from the culture medium or from the cells. In another embodiment, a culture of the cyanobacterial cells is grown in an indoor photobioreactor system.

[00185] In another embodiment, the method for producing a genetically enhanced *Cyanobacterium* sp. host cell uses an extrachromosomal plasmid derived from an endogenous plasmid of the host cell to introduce a recombinant nucleic acid sequence into the host cell. This endogenous plasmid can be, for example, an extrachromosomal plasmid derived from the 6.8 kb endogenous plasmid of ABICyano1.

[00186] In an embodiment, the cyanobacterial strain ABICyano1 is transformed with at least one recombinant gene in order to produce the desired MAA. It has been found that the use of modified endogenous plasmids improves the stability of the plasmid in the host cell.

[00187] The cyanobacterial strain ABICyano1 contains three endogenous plasmids. In combination with other genotypic and phenotypic attributes, these endogenous plasmids differentiate ABICyano1 from other cyanobacterial species. One plasmid is 6,826 base pairs, another is 39,702 base pairs, and a third plasmid is 28,554 base pairs. The 6,826 bp endogenous plasmid is alternatively referred to herein as pABICyano1, p6.8 or 6.8. As disclosed herein, plasmid 6.8 has been modified *in vivo* and *in vitro* for use as a plasmid vector containing genes of interest for the production of compounds of interest. In an embodiment, a modified endogenous vector derived from p6.8 from ABICyano1was developed. The modified endogenous vector from ABICyano1 can be used to transform cyanobacteria from a broad range of genera, including ABICyano1 itself, as described in the PCT application WO 2014/100799 A2 which is hereby incorporated by reference.

[00188] In an embodiment, the cyanobacterial strain ABICyano4 is transformed with the recombinant genes in order to produce the desired MAA. It has been found that the integration of recombinant genes into the endogenous plasmids improves expression of the respective enzymes. The cyanobacterial strain ABCyano4 comprises three endogenous plasmids, which, in combination with other genotypic and phenotypic attributes, differentiate ABICyano4 from other *Cyanobacterium* species. One plasmid herein termed "pABCyano4B" is 37990 base pairs. A

second, smaller plasmid "pABCyano4C" is 31678 base pairs. In an embodiment, both plasmids can be used for integration of recombinant genes via homologous recombination.

[00189] Several exemplary plasmids for integration of genes into the endogenous plasmids pABCyano4B and pABCyano4C of ABCyano4, respectively, carrying genes encoding enzymes to produce MAAs are listed below in Table **3A**. The plasmid maps of these constructs are shown in the Fig. as listed below in Table **3A**. Generated strains were transformed with at least one or various plasmids, respectively, as denoted below in table 3A. For example, strain AB1322 was transformed with plasmid #2865. In the left-hand column in Table 3A titled "*plasmid name*" one plasmid is named, the next column titled "*generated strain #*" list the denomination of a ABCyano4 strain transformed with this plasmid (and maybe additional plasmids), followed by the next column indicating the respective Fig. showing this plasmid and its SEQ ID NO.

Table 3A: Integrative plasmids for production of a MAAs in ABCyano4

Plasmid Name	Generated Strain #	Figure	SEQ ID NO:
#2865\oriVT-pABCyano4B_HFL1-PsmtA-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCyano4B_HFL2	AB1322 (plasmid #2865)	19	158
#2891\oriVT-pABCyano4C_int1-PsmtA-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCyano4C_int2	AB1333 (plasmids #2865 + #2891)	20	159
#2892\oriVT-pABCyano4C_int1-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCyano4C_int2	AB1334 (plasmids #2865 + #2892)	21	160
#2848\oriVT-ycf37_ABCyano4_up-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-ycf37_ABCyano4-down	AB1361 (plasmids #2865 + #2848)	18	161
#3094\oriVT-pABCC1710C_int1-PsmtA1710-Tery_2966-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4104 (plasmids #3094 + #3122)	42	183
3094\oriVT-pABCC1710C_int1-PsmtA1710-Tery_2966-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4105 (plasmids #3094 + #3123)	42	183
#2995\oriVT-PcpcB1710_up-FRT-PrbcL-aadA**-TB0014-FRT-PoprB1710'-UTRcpcB1710-cpcB1710_down	AB4014 (plasmids #2865 + #2848 + #2995)	22	162
#2991\oriVT-pABCyano4C_int1_HFL1-PsmtA-Ava_3858(ABICyano1opt)-PrbcL*-NpF5597(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCyano4C_int2	AB4028 (plasmids #2865 + #2991)	43	188

#3050\oriVT-pABCC1710C_int1-PsmtA-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PcpcB-Km**-TB0014-pABCC1710C_int2	AB4060 (plasmids #2865 + #2848 + #2995 + #3050)	23	163
#3075\oriVT-pABCC1710C_int1_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-PrbcL1710*-mysD_HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4068 (plasmids #2865 + #3075)	24	164
#3125\oriVT-pABCC1710C_int1-PsmtA1710-mysA_HL-69(AB1opt)-PsmtA1710-mysD_HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4094 (plasmids #2865 + #3125)	30	165
#3123\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-NpF5597(AB1opt)-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4100 (plasmids #2991 + #3123)	29	166
#3125\oriVT-pABCC1710C_int1-PsmtA1710-mysA_HL-69(AB1opt)-PsmtA1710-mysD_HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4101 (plasmids #2865 + #2848 + #2995 + #3125)	30	165
#3113\oriVT-pABCC1710C_int1-PsmtA-ap3855(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4102 (plasmids #2865 + #2848 + #2995 + #3113)	31	167
#3075\oriVT-pABCC1710C_int1_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-PrbcL1710*-mysD_HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4103 (plasmids #2865 + #2848 + #2995 + #3075)	24	164
#3122\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4104 (plasmids #3094 + #3122)	28	168
#3123\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-NpF5597(AB1opt)-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4105 (plasmids #3094 + #3123)	29	166
#3094\oriVT-pABCC1710C_int1-PsmtA1710-Tery_2966(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4075 (plasmids #2865 + #3094)	46	183
#3095\oriVT-pABCC1710C_int1-PsmtA1710-OMT_TS821(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4075 (plasmids #2865 + #3095)	25	169

#3096\oriVT-pABCC1710C_int1-PsmtA1710-OMT_PCC7335(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4076 (plasmids #2865 + #3096)	26	170
#3110\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA1710-OMT_KZN001(ABICyano1opt1)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710B_HFL2	AB4090 (plasmid #3110)	27	171
#3140\oriVT-pABCC1710C_int1-PsmtA1710-Ava_3858(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C_int2	AB4111 (plasmids #2865 + #2848 + #2995 + #3140)	32	172
#3122\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4131 (plasmids #3096 + #3122)	28	168
#3182\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-mysD1_IMS101(ABICyano1opt)-TpsaB-pABCC1710B_HFL2	AB4133 (plasmids #3096 + #3182)	44	189
#3183\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA1710-mysD2_IMS101(ABICyano1opt)-TpsaB-pABCC1710B_HFL2	AB4134 (plasmids #3096 + #3183)	45	186
#3190\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(AB1opt)-IScpcBA*1-Ava_3857(AB1opt)-IScpcBA*1-Ava_3856(AB1opt)-TpsaB-PsmtA-cas_NIES2100(AB1opt)-TpsaB-PrbcL-Gm**-TB0014*-pABCC1710B_HFL2	AB4140 (plasmids #2892 + #3190)	33	173
#3123\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-NpF5597(AB1opt)-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4163 (plasmids #3096 + #3123)	29	166
#3211\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-	AB4164 (plasmids #3096 + #3211)	46	187

PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-Tery_4684-IScpcBA*1-Tery_2968(ABICyano1opt)-TpsaB-pABCC1710B_HFL3			
#3211\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-Tery_4684-IScpcBA*1-Tery_2968(ABICyano1opt)-TpsaB-pABCC1710B_HFL3	AB4165 (plasmids #3094 + #3211)	46	187
#3182\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-mysD1_IMS101(ABICyano1opt)-TpsaB-pABCC1710B_HFL2	AB4168 (plasmids #3094 + #3182)	44	185
#3183\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA1710-mysD2_IMS101(ABICyano1opt)-TpsaB-pABCC1710B_HFL2	AB4169 (plasmids #3094 + #3183)	49	186
#3213\oriVT-pABCC1710C_int1-PsmtA1710-Ava_3858(ABICyano1opt)-linkerX-Ava_3857(AB1opt)-PrbcL1710*-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-FRT-pABCC1710C_int2	AB4179 (plasmids #3123 + #3213)	34	174
#3214\oriVT-pABCC1710C_int1-PsmtA1710-Ava_3858(ABICyano1opt)-linkerY-Ava_3857(ABICyano1opt)-PrbcL1710*-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-FRT-pABCC1710C_int2	AB4181 (plasmids #3123 + #3214)	35	175
#3287\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-mysD_K005(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4182 (plasmids #3094 + #3287)	47	188
#3287\oriVT-pABCC1710B_HFL1-PsmtA1710-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TpsaB-PsmtA-mysD_K005(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B_HFL2	AB4183 (plasmids #3096 + #3287)	47	188
#3289\oriVT-pABCC1710B_HFL1-PsmtA1710-mysA_HL-69(AB1opt)-IScpcBA*1-mysB_HL-		48	191

69(AB1opt)-IScpcBA*1-mysC-HL-69(AB1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCC1710B_HFL2			

[00190] Several exemplary plasmids for expression of MAA synthesis genes in *Escherichia coli* are listed below in Table **3B**. The plasmid maps of these constructs are shown in Fig. as described in table **3B.** Table **3D** describes the various abbreviations used for describing the plasmids.

Table 3B: Plasmids for production of a MAAs in *Escherichia coli*

Plasmid Name	Generated Strain #	Figure	SEQ ID NO:
#3130\pJet1.2::lacI-Ptac-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TB0011-Ptac-ap3855(ABICyano1opt)-TpsaB	BL21 #3130	36	176
#3131\pJet1.2::lacI-Ptac-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TB0011-Ptac-NpF5597(ABICyano1opt)-TpsaB	BL21 #3131	37	177
#3186\pJet1.2::lacI-Ptac-Ava_3858(ABICyano1opt)-IScpcBA*1-Ava_3857(ABICyano1opt)-IScpcBA*1-Ava_3856(ABICyano1opt)-TB0011-Ptac-Tery_2966(ABICyano1opt)-TpsaB-Ptac-sfp_NIES2100(ABICyano1opt)-IScpcBA*1-NRPS_NIES2100(ABICyano1opt)-TpsaB	BL21 #3186	38	178

Table 3C: List explaining the abbreviations

aadA**	Codon usage adapted aminoglycoside resistance protein (spectinomycin resistance)
ABICyano1opt	Codon usage adapted to ABICyano1 version 1
IScpcBA*1	Modified intergenic sequence of cpcBA operon
TpsaB	Terminator sequence from psaB
pABICyano1B_Rep	Replication region of the endogenous plasmid pABICyano1B
Gm**	Gentamycin resistance codon adapted to ABICyano1
TB0014, TB0014*, TB1002, TT7, Toop*1	Terminator sequence
pABCyano4B_HFL1	Homologous upstream flanking region for pABCyano4B
pABCyano4B_HFL2	Homologous downstream flanking region for pABCyano4B
ble(ABICyano1opt)	Bleomycin resistance codon adapted to ABICyano1
pABCyano4C_int1	Homologous upstream flanking region for pABCyano4C
pABCyano4C_int2	Homologous downstream flanking region for pABCyano4C
Km**	Kanamycin resistance codon adapted to ABICyano1
FRT	Binding site of flippase
flp	Flippase coding gene
ABCyano4opt1	Codon usage adapted to ABCyano4 version 1
pJet1.2::lacI-Ptac	Plasmid pJet1.2 harboring the lacI repressor and Ptac promoter (IPTG inducible)

EXAMPLES

Example 1

General Methods

[00191] Restriction endonucleases were purchased from Thermo Fisher Scientific, unless otherwise noted. PCR was performed using a Biometra thermocycler (Biometra, Germany), using Phire Plant Direct PCR Master Mix polymerase or Taq DNA polymerase (Thermo Fisher Scientific) for diagnostic amplifications, and Phusion polymerase for high fidelity amplifications. PCR temperature profiles were set up as recommended by the polymerase manufacturer. Cloning was performed in *E. coli* using NEB10-beta or NEB Turbo competent cells (New England Biolabs) following the manufacturer's protocol. NEBuilder HiFi assembly kits were purchased from New England Biolabs. Gene synthesis was conducted by GeneArt Gene Synthesis (Thermo Fisher Scientific).

[00192] *E. coli* strains HB101 (Promega), NEB10 and NEB Turbo (New England Biolabs), andEC100D (Epicentre)) were grown in Luria-Bertani (LB) medium at 37 °C. carbencillin (100 μg/mL), kanamycin (50 μg/mL), and chloramphenicol (34 μg/mL), zeocin (25 μg/ml), streptomycin (25 μg/ml) were used when appropriate. Cultures were continuously shaken overnight at 200 rpm and at 100 rpm when used for conjugation. ABICyano1 and ABCyano4 were cultured at from 28 °C to 37 °C in liquid BG11 fresh water medium in bubbled bottles under continuous illumination or 12h/12h day/night cycles of approximately 30 – 40 μmol photons*m^{-2} *sec^{-1}.

[00193] Plasmid DNA from *E. coli* strains was isolated using a GeneJet Plasmid Miniprep Kit (Fermentas) according to the manufacture's protocol. For plasmid isolation from putative ABICyano1 transformants, total DNA was prepared according to Saha et al. (2005), *World Jour. Microbiol Biotechnol* 21:877-881.

Example 2

Preparation of Cyanobacterial Culture Medium

[00194] For cyanobacterial growth, BG-11 stock solution was purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO). Marine BG-11 (MBG-11) was prepared by dissolving 35 g Instant Ocean (United Pet Group, Inc, Cincinnati, OH) in 1 L water

and supplementing with BG-11 stock solution. Vitamin B12 (Sigma Aldrich) was supplemented to MBG-11 to achieve a final concentration of 1 μg/L, as needed. Stock solutions of the antibiotics were purchased from Sigma Aldrich or Carl Roth GmbH).

[00195] Unless otherwise noted, the ABICyano1 and ABCyano4 transformants were selected on solid BG11 medium containing 10 - 20 μg/mL of the appropriate antibiotic. The choice of culture medium can depend on the cyanobacterial species. In an embodiment of the invention, the following BG11 medium for growing cyanobacteria can be used. When salt water species are grown, artificial seawater recipe (Table 4) is used to prepare the culture medium to yield artificial seawater medium BG11, denoted "ASW BG11".

Table 4: Culture Medium

BG11 Medium	
Compound	**Amount**
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g
Trace metal mix A5	1.0 ml
Distilled water	1.0 L
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Composition of Trace Metal Mix	
Compound	**Amount**
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L
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Composition of artificial seawater	
Chemical Components	**g / 1L**
Sodium Chloride	25.84
Magnesium Sulfate . 7 H20	6.36
Magnesium(II) Chloride . 6 H20	5.06
Potassium Chloride	0.62
Calcium Chloride . 2 H20	1.36
Salinity Measured (psu)	34 psu

Example 3

Transformation of ABICyano1 with p6.8 kb Based Shuttle Vector

[00196] The endogenous 6.8 kb plasmid of ABICyano1 (p6.8) was used as a means of shuttling exogenous DNA to cyanobacterial host cells. By inserting an origin of replication that is effective in *E. coli* (such as R6Kori or oriVT), the p6.8 kb plasmid DNA was manipulated in bacteria, such as *E. coli*, to incorporate genes and sequences of interest into a recombinant p6.8 kb. Further, modifications to decrease the effectiveness of endogenous restriction systems that are present in ABICyano1, such as methylation, can be performed.

[00197] The presence of an origin of replication that is already on ABICyano1 can assist with replication of the recombinant p6.8 kb once it is transferred into a host cell. Multiple cloning sites can be added to allow for several different antibiotic resistance genes to be added, if desired. Multiple cloning sites can also be inserted to allow for ease of insertion of various expression cassettes. In this way, various sequence segments of the plasmid can be replaced with other sequence segments as needed.

Example 4

Competent ABICyano1 Cells for Transformation by Conjugation

[00198] The following method was used to treat cells of the strain *Cyanobacterium* sp. ABICyano1 prior to conjugation. The method involves several steps: treatment of cells with N-acetylcysteine (NAC), washing steps that utilize NaCl, treatment with lysozyme, and subsequent washing followed by a conjugation procedure.

[00199] Two hundred mL of an exponentially growing culture (OD_{750nm} greater than about 0.5 and less than about 1.0) was incubated with NAC for 2 days at 16 °C (end concentration of NAC is about 0.1 mg/mL) without shaking. This pretreatment was followed by several steps to weaken the cell wall. The pretreated culture was pelleted at 4400 rpm and washed with 0.9% NaCl containing 8 mM EDTA.

For further treatment with lysozyme, the cell pellet was resuspended in 0.5 M sucrose and incubated 60 minutes at room temperature (RT) with slow shaking (85 rpm). Then, cells were centrifuged and resuspended in 40 mL of a solution containing 50mM Tris (pH 8.0), 10 mM EDTA (pH 8.0), 4% sucrose, and 20-40 µg/mL lysozyme. After incubation at rt for 10-15 minutes, cells were centrifuged and washed three times using different washing solutions; i) 30 mM Tris containing 4% sucrose and 1 mM EDTA; ii) 100 mM Tris containing 2 % sucrose and iii) with BG11 medium. All centrifugation steps before lysozyme treatment were performed at 4400 rpm for 10 minutes at 10 °C. All centrifugations after the lysozyme treatment were performed at 2400 rpm for 5 minutes at 4 °C. Resuspended cells were used for conjugation.

Example 5

Transformation of ABICyano1 by Conjugation

[00200] Gene transfer to ABICyano1 was performed using conjugation. Generated plasmids containing oriVT were used for conjugation. The shuttle vectors were transformed into ABICyano1 following a modified conjugation protocol which includes the pretreatment of ABICyano1 as described in the above Example.

~~[00201]~~ Triparental mating was performed as follows: *E. coli* strain J53 bearing a conjugative RP4 plasmid and E. coli strain HB101 bearing the cargo to be introduced into ABICyano1 and the pRL528 helper plasmid (for in vivo methylation) were used. *E. coli* strains were grown in LB broth supplemented with the appropriate antibiotics overnight at 37 °C with shaking at 100 rpm. An aliquot of 3 – 5 mL of each culture was centrifuged, washed twice with LB medium and resuspended in 200 µL LB medium. Subsequently, the *E. coli* strains were mixed, centrifuged and resuspended in 100 µL BG11 medium. Two hundred mL of exponentially growing cyanobacterial culture (OD_{750nm} of greater than 0.5 and less than 1.0) was centrifuged (3000 rpm, 10 minutes), pretreated as described in Example 4, and subsequently washed and resuspended in 400

μL BG11 culture medium containing Tris/sucrose buffer (Example 4). A 100 μL aliquot of resuspended cyanobacterial and *E. coli* cultures was mixed and applied onto a membrane filter (Millipore GVWP, 0.22 μm pore size) placed on the surface of solid BG11 medium supplemented with 5% LB. Petri dishes were incubated under dim light (5 $\mu E/m^2$-sec) for 2 days. Cells were then resuspended in fresh BG11 medium and plated onto selective medium containing 10 and 15 μg/mL kanamycin, respectively. The following selection conditions were used: light intensity of approximately 20 – 40 $\mu E/m^2$-sec at a temperature of approximately 28 °C. Transformants were visible after approximately 7-10 days. The transformant colonies were then plated on BG11 media containing 15 μg/mL kanamycin and then transferred stepwise to higher kanamycin concentrations (up to kanamycin 60 μg/mL) to aid in the selection process.

Example 6

Transformation of *Cyanobacterium sp.* ABICyano1 or ABCyano4 by Electroporation

[00202] Electroporation can also be used for successful transformation of *Cyanobacterium* sp. ABICyano1 and ABCyano4, or other strains such as *Arthrospira*, *Synechococcus*, and *Synechocystis*, using, for example, the same plasmids as for conjugation, but with lower efficiency.

[00203] As with the conjugation transformation protocol (above), strain-specific adaptations of standard electroporation protocols can be made to avoid DNA digestion by endogenous restriction enzymes and to allow DNA entry through the CPS layer. To achieve successful electroporation, DNA is protected against endogenous restriction enzymes by methylation. Prior to electroporation, ABICyano1 and ABCyano4 cells are pretreated with positively charged polyaminoacids such as poly-L-lysine hydrobromide or poly-L-ornithine hydrochloride or combinations thereof (in particular poly-L-lysine hydrobromide) in order to increase the DNA uptake efficiency.

[00204] As an example, 100 mL of exponentially growing ABICyano1 or ABCyano4 cultures (corresponding to a cell density of approximately $2x10^7$ cells/mL), were harvested, washed and resuspended in 0.9 % NaCl containing 25 mM Tris-HCl (pH 8.0). Poly-L-lysine hydrobromide was added to the resuspended cells to obtain a

final concentration of 50 µg/mL ABICyano1 or ABCyano4 cells were then incubated for several hours or overnight before electroporation.

[00205] In a typical procedure, 50 mL of poly-L-lysine hydrobromide treated ABICyano1 or ABCyano4 cells were harvested and treated with 30 mL ice-cold BG11 containing 6% DMSO. After incubation on ice for 20 minutes, cells were harvested and frozen in liquid nitrogen for 15 minutes. These pre-frozen cells were thawed by adding 15 mL ice-cold buffer containing 1 mM HEPES (pH7.5), 0.2 mM K_2HPO_4 and 0.2 mM $MgCl_2$. The cells were washed sequentially once more with 1 mM HEPES and ETMT buffer containing 0.1 mM HEPES, 0.2 mM K_2HPO_4 and 0.2 mM $MgCl_2$. The cells were harvested by centrifugation at 15000 x g for 5 minutes. All of the washes and centrifugations were carried out on ice or in a pre-chilled centrifuge (4 °C). For each electroporation procedure, 3 µg methylated DNA is added to 100 µL of concentrated cells. Cells were electroporated in a cuvette with a 2 mm gap between the electrodes and pulsed once in a Gene Pulse X-cell (Bio-Rad) using an exponential decay protocol (electric field strength of 8 kV/cm, capacitance of 25 µF, resistance of 400 ohms, for a time of approximately 8-9 ms). After electroporation, 1-2 mL BG11 medium was immediately added to the cyanobacterial suspension, which was subsequently transferred to a 50 mL flask containing 15 mL fresh BG11 medium. After incubation for 1- 2 days under normal light (30 – 40 µE/m^2-sec) with gentle shaking at 30 °C, recovered cultures were centrifuged, resuspended in 500 µL BG11 medium and placed onto selective media (BG11 containing 20 µg/mL Km or 40-60 µg/mL of spectinomycin).

Example 7

Transformation of *Cyanobacterium sp.* ABCyano4 by Conjugation

[00206] The following method was used for transformation of ABCyano4. An exponentially growing culture ($0.5 < OD_{750nm} < 3$) was pelleted at >4000 RCF and washed twice with 0.9% NaCl containing 8 mM EDTA and once with 0.75 M NaCl. Then, cells were centrifuged and resuspended in BG11 medium containing 10 mM TES pH 7.3.

[00207] Biparental mating was performed as follows. E. coli strain EC100D bearing a conjugative RP4 Δaph plasmid, the pRL528-based helper plasmid #2709 (pRL528-

lacI-Ptac-M.MspI-Toop) for in vivo methylation and the cargo plasmid to be introduced into *Cyanobacterium sp.* ABCyano4. *E. coli* strains were generally grown in LB broth supplemented with the appropriate antibiotics overnight at 37 °C with shaking at 100 rpm. An aliquot of 3–5 ml of each culture was centrifuged, washed twice with LB medium and resuspended in 200 µl LB medium. Subsequently, the *E. coli* strains were mixed, centrifuged and resuspended in 100 µl BG11 medium. A 100 µl aliquot of the resuspended cyanobacterial cells and the *E. coli* cultures was mixed and applied onto a membrane filter (Millipore GVWP, 0.22 µm pore size) placed on the surface of solid BG11 medium supplemented with 5% LB. Petri dishes were incubated under dim light of 5 µmol photons m^{-2} s^{-1} for two days. Cells were then resuspended in fresh BG11 medium and plated onto selective medium containing > 10 µg/ml of the respective antibiotic. The following selection conditions were used: light intensity approximately 20 – 40 µmol photons m^{-2} sec^{-1} at a temperature of approximately 30 °C. Transformants were visible after approximately 3-4 days. The transformant colonies were then stepwise transferred to higher antibiotic concentrations.

[00208] Integration of heterologous genes into endogenous plasmids of ABCyano4 was conducted via homologous recombination, a method well known to those skilled in the art. The integrative plasmids harbor an oriVT for conjugative transfer and homologous 2-3 kb flanking regions upstream and downstream of an integrative sequence followed by a resistance marker gene. In order to insert MAA synthesis genes into the endogenous plasmids pABCyano4B and pABCyano4C respective integrative regions were applied as shown in Fig. 40 and Fig. 41.

Example 8

Assessment of MAA production rates via GC vial tests

[00209] Strains were cultivated in 20 mL GC vials filled with 2 mL culture suspension using GC assay medium (ASW BG11 supplemented with 10 mM urea, 10 mM NaHCO3, buffered with TES at pH 7.3. The GC vials were placed on a rotary shaker at 450 rpm at 35°C and a 120 -200 µE m^{-2} s^{-1} homogenous light field from the bottom. Initially, 6 mL pure CO_2 was injected through the gas-tight lid. Pre-cultures were grown until an OD_{750nm} between 5 and 10 and then diluted to an OD set point of about 5 using fresh medium. For induction of MAA production ZnSO4 was added and

6 mL pure CO_2 was injected into each GC vial. Induced cultures were placed again on a rotatory shaker with 450 rpm at 120 -200 µE m^{-2} s^{-1} homogenous light field from the bottom. Experiments were performed in 2-3 biological replicates per strain. From each GC vial culture samples were taken daily and measured for growth (increase in optical density at 750nm) and MAA production. MAA concentration was determined spectrophotometrically using Lambert-Beer's law and known absorbance properties (molar extinction coefficient and molecular weight) of the various MAAs as described earlier.

Example 9

Assessment of MAA production rates, cell growth and carbon partitioning via cultivation in lab-scale vertical photobioreactor (LvPBRs)

[00210] Cultivations were performed in 1.2L LvPBRs in BG11 artificial seawater (35 psu). The cultures were cultivated at a 12h / 12h dark / light cycle illuminated with 350 µE m^{-2} s^{-1} from one side of the LvPBRs. During the night the temperature was 25 °C with a ramping to 37 °C during the day. The strains were cultivated at pH 7.3 ± 0.01, controlled by CO_2 (15 % CO_2 in air) injection into the liquid phase. Mixing of the culture was ensured by continuous aeration at 38 mL / minutes. The carbon-partitioning was determined by calculation from the OD measured at 750nm using an experimentally determined DW/OD ratio (1L ABCyano4 culture with an OD of 1 corresponds to 0.2g/L dry biomass). MAA concentration was determined spectrophotometrically using Lambert-Beer's law and known absorbance properties of the various MAAs (molar extinction coefficient and molecular weight) as described earlier.

Example 10

Scale-up of Modified Cyanobacteria to Produce an MAA

[00211] A culture of modified cyanobacteria is scaled-up to flasks, then 1 liter containers, then 5 liter containers, then to outdoor or indoor photobioreactors, in a suitable culture medium, with the pH set at 7.3 by use of air bubbling with CO_2 addition on demand. After 2 days, the host cells are induced to produce the MAA and production allowed to proceed for a specified period of time, usually not more than 30 days of continuous growth under production conditions.

Example 11

Isolation of MAA from Genetically Modified Cyanobacterial Host Cells

[00212] A culture of MAA-producing cyanobacterial cells is grown and the production of the MAA of interest is induced in an indoor or outdoor photobioreactor. After a selected time period of growth, the culture is harvested by centrifugation or other means, and the MAA is collected from the cell-free medium using crossflow ultrafiltration combined with bulk ion exchange chromatography, or alternatively through absorption/de-absorption cycling using an organic substrate such as activated charcoal as an isolation matrix. The concentrated MAA is quantified and further purified, if needed. If desired, other products of interest are isolated from the culture in addition to the MAA, such as biomass, pigments, proteins, lipids, etc.

[00213] Alternatively, MAAs can be isolated from the concentrated culture biomass. In this instance, the cell biomass is harvested by centrifugation or other means of harvesting, with the result that the cells are separated from the culture medium. The concentrated cell slurry is dried and the cells disrupted by grinding or other means. The cellular material is resuspended in water or 30%(v/v) ethanol in water, mixed for 30 minutes at room temperature, and centrifuged to separate the insoluble solid biomass from the soluble MAA. The MAA-enriched cell extract can then be used without further purification, or it can be further purified, such as, for example, using crossflow ultrafiltration, centrifugation, and ion exchange, as needed. The purity of the MAA is determined using mass spectrometry or by other means (i.e. photometrically in combination with a TOC analyzer).

Example 12

Quantitative Analysis of MAAs Using Capillary Electrophoresis and Hydrophilic Interaction Liquid Chromatography (HILIC)

[00214] The amount (and type) of MAA produced is quantified by Capillary Electrophoresis, following the method described in Hartmann et al. "Quantitative analysis of mycosporine-like amino acids in marine algae by capillary electrophoresis with diode-array detection", Jour. Pharm. Biomed. Analysis, 138:153-157 (2017).

[00215] Another suitable method for quantitation and analysis of MAAs is the use of HPLC, as described, for example, in Rastogi et al., Appl. Microbiol. 119:753-762 (2015). Hydrophilic interaction liquid chromatography (HILIC) is also utilized (Hartmann et al., *Mar. Drugs* 13:6291-6305 (2015).

[00216] The following set-up was used to conduct the hydrophilic interaction chromatography at room temperature. The column employed was a ZIC-HILIC, 150 X 2.1 mm, 3.5 µm, 200 Å, which contains a zwitterionic stationary phase covalently attached to porous silica. A SeQuant ZIC-HILIC Guard 20 X2.1 mm, coated with PEEK (Polyetheretherketon) was used as a guard column. A mixture Acetonitrile/ 5mM ammonium acetate in water 9:1 was used as a mobile phase A. A mixture of Acetonitrile/5mM ammonium acetate in water 1:1 was used as mobile phase B. The flow rate was 0.3 mL/minutes and the diode array detector (DAD) was set to an absorption maximum of 320 nm. The injection volume for each HILIC run was 50 µl. The sample was prepared by diluting the sample (culture medium ASW or BG11) to 1:10 or 1:5 with mobile phase A (Acetonitrile/ 5mM ammonium acetate in water 9:1). The mixture is then centrifuged to allow the separation of eventual precipitate (not always visible) and further filtered through a PTFE filter (0.45 µm) and finally applied on the HILIC column. The gradient elution mode was employed, starting with a mixture of 80 vol % mobile phase A and 20 vol % mobile phase B. The MAAs were eluted over a time period of 54 minutes employing the gradient as shown in Fig. 17. Fig. 17 shows the composition of the buffer during the gradient elution starting with 80 vol % mobile phase A and 20 vol % mobile phase B and then gradually increasing the fraction of mobile phase B until 100 vol % B are reached after 30 minutes. For 8 minutes 100 vol % B are maintained and subsequently within 1 minute the composition is gradually changed to of 80 vol % mobile phase A and 20 vol % mobile phase B.

[00217] The retention times in minutes and the absorption maxima of some of the MAAs are shown in the below table:

Table 5: Retention Times and Wavelength of Maximum Absorption for some MAAs:

MAA	Retention Time (minutes)	Max Abs (nm)
Mycosporine-glycine	7.5-7.7	309-310

Shinorine	11.4	333-334
Mycosporine-2-glycine	11.4	332-333
Porphyra-334	10.4	333-334
Palythine	9.6	320-321

Example 13

Preparation of a Skin Care Agent Using MAAs from Cyanobacteria

[00218] An MAA is produced in an indoor or outdoor photobioreactor containing modified cyanobacterial strain according to the above examples. The MAA is obtained from the culture, and is further purified using either a tangential cross-flow filtration unit or a batch addition of an ion exchange medium. The MAA obtained via ion exchange method has a purity of at least 95% by HPLC analysis. The MAA is further purified to about 99% using additional chromatography methods. The purified MAA is mixed with one or more different MAAs (of different absorbance maxima) that have also been prepared from cyanobacteria. This mixture of various MAAs increases the breadth of the UV protection over a wider absorbance range. The MAA mixture is then mixed with a naturally obtained, suitable carrier for application to human skin (such as an oil or a lotion) to result in a spreadable material that protects the skin from sun damage.

Example 14

Increase in MAA production by culturing cyanobacterial strains including a low content of antenna complexes and two mysA gene copies

[00219] ABCyano4 cells AB1322 transformed with the plasmid #2865 encoding the *Anabaena variabilis* mysA, mysB and mysC genes Ava_3858, Ava_3857 and Ava_3856 were further transformed with the plasmid #2892 encoding D-ala-D-ala ligase (Ap3855) from *Aphanothece halophytica* for mycosporine-2-glycine production resulting in the ABCyano4 strain AB1334. These AB1334 cells were used as reference strain in this cultivation experiment.

[00220] ABCyano4 cells AB4102 exhibiting a low content of both chlorophyll and phycocyanin in comparison to the wildtype cell were generated by transforming ABCyano4 cells AB1322 with the plasmid #2848 conferring a lower chlorophyll

content, the plasmid #2995 conferring a lower phycocyanin content and plasmid #3113 encoding D-ala-D-ala ligase (Ap3855) from *Aphanothece halophytica* for mycosporine-2-glycine production. ABCyano4 cells AB4111 which in comparison to AB4102 additionally include two mysA (Ava_3858) copies instead of one copy were generated by transforming AB1322 with the plasmids #2848, #2995 and plasmid #3140 encoding D-ala-D-ala ligase (Ap3855) from *Aphanothece halophytica* for mycosporine-2-glycine production and a further copy of the gene Ava_3858 (mysA). GC vial data were generated as described above. Fig. 2A shows that the strain AB4111 exhibits a lower growth (OD_{750nm}) compared to the reference strain AB1334 and the AB4102 cells only containing one mysA copy. However, as shown in Fig. 2B low pigment AB4111 cells with two mysA copies produce more mycosporine-2-glycine and also exhibit a higher carbon partitioning towards mycosporine-2-glycine in comparison to AB1334 and the AB4102 cells, when cultured for nearly 6 days.

[00221] Fig. 3A to Fig. 3C show the corresponding data for cell growth, mycosporine-2-glycine production and carbon partitioning for cells grown in LvPBRs. The cells were cultured in LvPBRs as mentioned above. Again, the AB4111 cells with two mysA (Ava_3858) copies produce the highest amount of mycosporine-2-glycine. Fig. 3D shows that in the strain AB4102 exhibiting a lower content of phycocyanin than strain AB1334 shows a reduced absorption at 615 nm, the absorption maximum of phycocyanin. Absorption at 680 nm for chlorophyll a is also reduced.

[00222] Genetically modified cyanobacterial ABCyano4 cells AB4094 producing shinorine/porphyra-334 were generated by transforming ABCyano1 cells AB1322 containing the *Anabaena variabilis* mysA, mysB and mysC genes (Ava_3858, Ava_3857 and Ava_3856) with plasmid #3125 including a second copy of the mysA gene from *Cyanobacterium stanieri* HL-69. This plasmid additionally includes the gene mysD from *Cyanobacterium stanieri* HL-69 encoding a D-alanine-D-alanine ligase for the production of shinorine/porphyra-334. These AB4094 cells served as a reference strain. ABCyano4 strain AB4101 with a low content of antenna complexes was transformed with all the plasmids of strain AB4094. AB4101 furthermore exhibits a lower chlorophyll content owing to transformation with plasmid #2848, and a lower phycocyanin content due to transformation with plasmid #2995. Fig. 4A, 4B and 4C show that AB4101 with two copies of mysA and a low content of chlorophyll and

phycocyanin antenna complexes in comparison to the wildtype strain produces more shinorine/porphyra-334 than AB4094 (Fig. 4B), but exhibits a similar growth as assessed by the $OD_{750\ nm}$ (Fig. 4A). The carbon-partitioning towards the production of shinorine/porphyra-334 is also higher for AB4101 (Fig. 4C).

[00223] The corresponding experimental data for LvPBR cultivations for both strains AB4094 and AB4101 are shown in Fig. 5A to 5D. These Fig. indicate again that the production rates for shinorine/porphyra-334 are higher for the AB4101 strain exhibiting a low chlorophyll and phycocyanin content compared to AB4094. Fig. 5D shows that the absorption intensity at 615 nm (phycocyanin) and 680 nm (chlorophyll) is reduced for AB4101 compared to AB4094 due to the lower content of both antenna complexes.

[00224] Fig. 6A to Fig. 6D present the cell growth, the shinorine/porphyra-334 production, carbon partitioning and the whole cell absorbance spectra for the ABCyano4 strains AB4068 and AB4103 obtained by LvPBR cultivations. AB4068 exhibits a wildtype level of antenna complexes and is able to produce shinorine/porphyra-334 due to integration of plasmid #2865 containing *Anabaena variabilis* mysA, mysB and mysC genes (Ava_3858, Ava_3857 and Ava_3856) and the integration of plasmid #3075 including a second copy of Ava_3858 and the gene mysD of HL_69. In addition to AB4068 the strain AB4103 was transformed with plasmids #2848 and #2995 conferring a lower chlorophyll and phycocyanin content. Fig. 6A shows that the cell growth for both strains is comparable until day 12 of the cultivation with AB4103 performing better from that point on to day 25 of the cultivation. Fig. 6B and 6C demonstrate that the shinorine/porphyra-334 production and the carbon partitioning towards shinorine/porphyra-334 is better for the low pigment strain AB4103. Fig. 6D demonstrates the reduced absorption at 615 nm for phycocyanin and at 680 nm for chlorophyll for the strain AB4103.

[00225] Fig. 7A evidences that a higher amount of MAA of the low pigment strain AB4103 stays associated with the biomass (MAA content in dry weight) compared to the strain AB4068 exhibiting a wildtype level of the antenna complexes chlorophyll and phycocyanin. Consequently, Fig. 7B shows that initially all the MAAs produced in the cell remain in or at the cell during day 1 (100% cell-associated MAA) and subsequently the amount of cell associated MAA decreases over the course of the cultivation until

day 25, because a fraction of the MAAs is released into the cultivation medium. However, even after day 25 of the cultivation around 70% of the MAAs were cell-associated for the low pigment strain AB4103, whereas only around 40% were found to be cell-associated for the strain AB4068. Therefore, the purification of the MAAs produced by the low pigment strains by the way of separating the biomass from the culture medium via centrifugation is much easier compared to the wildtype level pigment strains. In Fig. 7C and 7D the same trend is visible for the strains AB4094 and AB4101. Fig. 7C evidences that a higher amount of MAA of the low pigment strain AB4101 stays associated with the biomass (MAA content in dry weight) compared to the strain AB4094 exhibiting a wildtype level of the antenna complexes chlorophyll and phycocyanin. Fig. 7D illustrates that after day 20 of the cultivation more than 60% of the MAAs were cell-associated for the low pigment strain AB4101, whereas less than 50% were found to be cell-associated for the strain AB4094 with wild-type pigmentation.

Example 15

Increase in MAA production by culturing cyanobacterial strains including a MysAB fusion protein

[00226] Fig. 8A and Fig. 8B depict the general principle of increasing the production of MAAs by introducing a MysAB fusion protein in order to allow a "*substrate channeling effect*" taking place, leading to a higher production of MAAs. This "*substrate channeling effect*" due to the presence of a MysAB fusion protein allows the rapid conversion of the intermediate desmethyl-4-deoxygadusol produced by MysA into 4-deoxygadusol by the enzyme MysB, because both enzymes are in close proximity in the fusion protein. The ABCyano4 strain AB4100 serves as a reference strain and was transformed with the plasmids #2991 including the genes Ava_3858 (mysA) and NpF5597 (mysD) and the plasmid #3123 including the genes mysA, mysB and mysC (AvA_3858, AvA_3857, and AvA_3856) without including MysAB gene fusion. The ABCyano4 strain AB4179 was transformed with the same plasmid #3123 as reference strain AB4100, but not the plasmid #2991. Instead, AB4179 was transformed with the plasmid #3213 encoding a MysAB fusion protein, wherein MysA and MysB are connected via the so-called "*linker X*" and additionally containing NpF5597

(NP_mysD). A second ABCyano4 strain AB4181 was generated by transforming ABCyano4 with the plasmids #3123 and #3214. Plasmid #3214 includes the genes coding for a MysAB fusion protein, wherein MysA and MysB are connected via the so called "*linker Y*" and the plasmid additionally contains a gene coding for NpF5597 (MysD). Fig. 8A also shows the amino acid sequences of both peptide linkers, "*linker X*" and "*linker Y*". Fig. 8B represents the differences in the expression of the various proteins between the reference strain AB4100 and the two strains AB4179 and AB4181 both expressing the recombinant MysAB fusion protein. Both strains AB4179 and AB4181 also include genes coding for the single proteins MysA and MysB. This approach ensures, that the formation of large aggregates of MysAB fusion protein multimers, which might be enzymatically inactive, can be avoided. For example, the protein encoded by the gene mysA is known to form a dimer (ACS Chem Biol. 2017 Apr 21;12(4):979-988). The concomitant expression of both the MysAB fusion protein, as well as the single MysA proteins enables the formation of dimers of for example the MysAB fusion protein together with single MysA monomers, which are enzymatically active as evidenced by the above discussed experimental data.

[00227] Fig. 9A, 9B and 9C depict the cell growth (OD_{750nm}), the formation of shinorine/porphyra-334 (Fig. 9B) and the carbon partitioning (Fig. 9C) for the reference strain and for both strains overexpressing the recombinant MysAB fusion protein. The strains overexpressing the recombinant MysAB fusion protein, AB4179 and AB4181 exhibit improved properties with regard to the growth and up to 30 % improved production rate for shinorine/porphyra-334 compared to the reference strain. Similarly, the carbon partitioning towards shinorine/porphyra-334 is also improved for the strains expressing the recombinant MysAB fusion proteins.

Example 16

Production of new MAA compounds Tery-347.1, Tery-347.2 and Tery-347.3

[00228] Fig. 10 depicts different proposed pathways for producing the novel mycosporine-like amino acids Tery-347.1, Tery-347.2 and Tery-347.3 employing different enzymes. It is possible that the steps denoted "*1*" and "*2*" in Fig. 10 take place in reverse order, because the O-methyltransferase is also able to convert mycosporine glycine into Tery-322. According to the proposed pathways labeled with "*1*",

mycosporine-glycine serves as a precursor for all 3 different novel MAAs Tery-347.1, Tery-347.2 and Tery-347.3. This compound can be converted into mycosporine-2-glycine by the enzyme Tery_2971 (MysD1) or Ap_MysD. The enzyme Tery_2970 (MysD2) converts mycosporine-glycine into shinorine (>90%). The enzymes NpF5597 of *Nostoc punctiforme* (Np_MysD) or the enzyme MysD_HL-69 [*Cyanobacterium stanieri* HL-69] can convert mycosporine-glycine into a mixture of shinorine/porphyra-334 by using either serine or threonine (Np_MysD with a ratio of about 60%:40% shinorine/porphyra-334 and MysD_HL-69 with a ratio of about 40%:60% shinorine/porphyra-334. The gene Nv_mysD encoding a D-alanine-D-alanine ligase MysD from *Nostoc verrucosum* KU005 can convert mycosporine-glycine into porphyra-334 by using threonine (>90%). The O-methyltransferase Tery_2966 can convert these intermediate compounds mycosporine-2-glycine, or shinorine/porphyra-334 into the novel mycosporine like amino acids Tery-347.1, Tery-347.2 and Tery-347.3 (step "*2*"), which all show an absorption maximum at 347 nm. These novel compounds all exhibit different retention characteristics in a Hydrophobic Interaction Liquid Chromatography (HILIC) indicating different chemical structures for these MAAs as shown in the following.

[00229] Fig. 11 depicts a hydrophobic interaction liquid chromatogram of the MAAs isolated from the ABCyano4 strain AB1333, which lacks the O-methyltransferase Tery_2966 and which was transformed with the plasmids #2865 and #2891, which were already described in more detail above. This ABCyano4 strain produces a mixture of shinorine/porphyra-334 and serves as a reference strain for the ABCyano4 strain AB4105 producing the novel mycosporine like amino acids Tery-347.1 and Tery-347.2.

[00230] Fig. 12 shows a hydrophobic interaction liquid chromatogram of the MAAs isolated from the ABCyano4 strain AB4105, which produces a mixture of Tery-347.1 and Tery-347.2. This ABCyano4 strain was transformed with the plasmids #3094, including a gene coding for Tery_2966 and the plasmid #3123, which encodes the enzymes for MAA production Ava_3858, Ava_3857, and Ava_3856 (MysA to MysC) and additionally the NpF5597 of *Nostoc punctiforme* (Np_MysD). The main peaks eluted from the HILIC column are denoted as "*347 nm_Tery_347.1*", "*347 nm_Tery_347.2*", "*334 nm_Shinorine*", "*334 nm_porphyra-334*" and "*334 nm_M-Gly-Ala*", indicating the absorption maxima of the respective components and their chemical

structure, if known. As indicated by the first arrow in Fig. 12, the peak being assigned to porphyra-334 is dramatically reduced in comparison to the chromatogram shown in Fig. 11 and a new peak, which could be assigned to the novel MAA compound Tery-347.1 appears in the chromatogram. Similarly, the peak belonging to shinorine is dramatically reduced in the chromatogram, whereas a new peak appears, which can be assigned to the novel compound Tery-347.2, as indicated by the second arrow. This clearly indicates that the novel MAA compound Tery-347.1 is produced from porphyra-334 and that the novel compound MAA Tery-347.2 is produced from shinorine. The fact that Tery-347.2 is produced from shinorine is known from another ABcyano4 strain AB4169, including a fourth gene encoding for the fourth enzyme Tery_2970, catalyzing the formation of shinorine (>90%), this strain additionally being transformed with a sixth gene encoding the sixth enzyme Tery_2966. This AB4169 strain produces mainly Tery-347.2 so that it is clear that shinorine is further converted to Tery-347.2 by the enzyme Tery_2966. Tery-347.1 exhibits a retention time of 8.994 minutes, whereas the other MAA compound Tery-347.2 has a retention time of 10.256 minutes, indicating different hydrophobicities and therefore different chemical structures for both compounds. The compound Tery-347.1 is supposed to be a methylated porphyra-334 compound, whereas the compound Tery-347.2 is a methylated shinorine compound. The ABCyano4 strain AB4105 was shown to also produce another novel MAA compound Tery-347.4, which is a methylated mycosporine-glycine-alanine with the molecular formula $C_{14}H_{22}N_2O_7$ as described herein.

[00231] Fig. 13A shows the hydrophobic interaction chromatogram of the MAA compounds produced by the ABCyano4 strain AB4104. This ABCyano4 strain was transformed with the plasmids #3122 including the genes mysA, mysB and mysC (Ava_3858, Ava_3857, and Ava_3856), as well as Ap_mysD (ap3855) and which additionally was transformed with the plasmid #3094 including the gene coding for the O-methyltransferase Tery_2966. The HILIC chromatogram shows that the compound Tery-347.3, which is a methylated mycosporine-2-glycine was formed from mycosporine-2-glycine. At the same time, the compound Tery-322 with an absorption maximum at 322 nm was also formed. This novel compound Tery-347.3 exhibits a retention time of 10.110 minutes, which is different to the novel MAA compounds described above, Tery-347.1 and Tery-347.2. These novel MAA compounds exhibit

different absorption characteristics in comparison to the other MAA compounds disclosed in this application or MAA compounds known from the literature and therefore can be used as UV-absorbing compounds with characteristics different to known MAAs.

[00232] Fig. 13B depicts an HPLC spectrum of the main MAA compound produced by the *E. coli* strain #3186, which is an *E. coli* BL21 strain transformed with the plasmid #3186 containing the genes for mysA, mysB, and mysC (Ava_3858, Ava_3857, and Ava_3856), the gene encoding Tery_2966 and a further gene encoding a nonribosomal peptide synthetase from *Calothrix sp.* NIES-2100 (SEQ ID NO. 193). This *E. coli* strain produces a new MAA compound having an absorption maximum at 347 nm, called Tery-347.x. It is currently not known whether this compound is Tery-347.1, Tery-347.2 or Tery-347.3 or a different MAA compound, structurally distinct from any of these MAA compounds with an absorbance maximum at 347nm.

Example 17

Alternative enzymes for the production of MAA compounds having a low homology to enzymes from *Trichodesmium erythraeum*

[00233] New O-methyltransferases-like enzymes were found employing a BLAST search with the NCBI database, which have a low sequence homology of less than 70% to the known O-methyltransferase Tery_2966, a Hydroxyneurosporene-O-methyltransferase from *Trichodesmium erythraeum* IMS101 (GenBank: ABG52124.1), which can be used to produce the novel MAA compounds Tery-347.1, Tery-347.2, Tery-347.3, Tery.347.x and Tery-322, which also is a novel MAA compound having an absorption maximum at 322 nm and which is a methylated mycosporine-glycine. Similarly, clavaminic acid synthetase-like enzymes could be identified, having a low sequence homology of less than 70% to the enzyme Tery_2972 from *Trichodesmium erythraeum* IMS101 (GenBank: ABG52130.1). These low sequence homology enzymes can for example be used for the conversion of mycosporine-2-glycine into palythine. Furthermore, new non-ribosomal peptide synthetases (NRPS) were identified having a sequence homology of less than 70% to the NRPS Tery_2968 from *Trichodesmium erythraeum* IMS101 (GenBank: ABG52126.1), which for example can be used to

convert Tery-322 into Tery-364, another unknown MAA compound having an absorption maximum at 364 nm. These novel low homology enzymes are involved in pathways for MAA production, which are shown in Fig. 39. The following experimental results will present evidences that these low homology enzymes can be used for the production of the same MAA compounds as the enzymes encoded by the genes Tery_2966, Tery_2968 and Tery_2972.

[00234] Fig. 14 depicts the UV-absorbance spectra of the main MAA compound Tery-322 produced by the ABCyano4 strain AB4075, expressing the O-methyltransferase from *Chroococcidiopsis* sp. TS-821 (SEQ ID NO: 86), the strain AB4076, including the O-methyltransferase from *Synechococcus* sp. PCC 7335 (SEQ ID NO: 92), and the strain AB4090, including the O-methyltransferase from *Euhalothece* sp. KZN 001 (SEQ ID NO: 88). For all strains the UV-absorbance spectra of two different clones are shown in Fig. 14. These O-methyltransferases exhibit a low sequence homology to the O-methyltransferase Tery_2966, but nevertheless all produce Tery-322, having an absorption maximum at 322 nm, because the MAA product of the ABCyano4 strain AB4074, expressing Tery_2966 shows the same absorption maximum.

[00235] Fig. 15A shows the HPLC profile of the main MAA compound produced by the ABCyano4 strain AB4075. This ABCyano4 strain was transformed with the plasmid #2865 containing the genes mysA, mysB, and mysC (Ava_3858, Ava_3857, and Ava_3856) for the production of mycosporine-like-glycine. Furthermore, this strain was also transformed with the integrative plasmid #3095 containing the gene encoding the O-methyltransferase from *Chroococcidiopsis sp.* TS-821 (SEQ ID NO: 86), a low sequence homolog having only 58% sequence identity with Tery_2966. The HPLC profile shows that the new MAA compound Tery-322 is produced and that therefore the same product is formed as when using Tery_2966. Thus, it is clear that the O-methyltransferase from *Chroococcidiopsis* sp. TS-821 can catalyze the same chemical reaction as Tery_2966. Fig. 15B shows the chromatogram of the main MAA compound produced by the ABCyano4 strain AB4076. This strain contains the O-methyltransferase from *Synechococcus* sp. PCC 7335 instead of the O-methyltransferase from *Chroococcidiopsis* sp. TS-821. The O-methyltransferase from *Synechococcus* sp. PCC 7335 has only 49% sequence identity with the O-

methyltransferase Tery_2966. The same compound Tery-322 is also produced by the O-methyltransferase from *Synechococcus* sp. PCC 7335, thereby evidencing that the O-methyltransferase from *Synechococcus* sp. PCC 7335 can catalyze the same chemical reaction as Tery_2966.

[00236] Fig. 16 shows the HPLC profile of palythine, the MAA compound produced from mycosporine-2-glycine by the ABCyano4 strain AB4140. This ABCyano4 strain was transformed with the plasmid #2892, already described above and additionally was transformed with the plasmid #3190, containing the genes mysA, mysB, and mysC (Ava_3858, Ava_3857, and Ava_3856) and the gene encoding the clavaminic acid synthetase from *Calothrix* sp. NIES-2100, an enzyme having only 61% sequence identity with the enzyme Tery_2972. Despite the low sequence identity, both enzymes Tery_2972 and the clavaminic acid synthetase from *Calothrix* sp. NIES-2100 catalyze the same reaction.

Example 18

Determination of the molecular weight and confirmation of the molecular formulas for the novel MAA compounds Tery-347.1, Tery-347.2, Tery-347.3 and Tery-347.4

[00237] Samples of Tery-347.1, Tery-347.2, Tery-347.3 and Tery-347.4 were analyzed using HILIC-MS/MS on a Waters Aquity UPLC equipped with a Merck-Sequant ZIC-cHILIC column using acetonitrile and ammonium acetate as running buffers. MS/MS analysis was performed in a data dependent mode on a Sciex 5600 TripleTOF mass spectrometer equipped with an electrospray source and automatic calibration system. For all novel MAA compounds, the molecular weight, and the molecular formulas as disclosed herein could be determined.

[00238] Articles, patents and other published literature referred to herein is incorporated by reference. Although the present disclosure has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

CLAIMS

What is Claimed is:

1. A genetically modified cyanobacterial cell for the production of mycosporine glycine exhibiting a low pigment phenotype compared to the wildtype with respect to the chlorophyll and the phycocyanin content of the cell, further comprising

- at least two copies of a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 80 % sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 (Ava_3858) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (3-dehydroquinate synthase MysA_HL-69),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 (Ava-3857) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (MysB_HL-69), and

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 (Ava-3856) or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC_HL-69).

2. The genetically modified cyanobacterial cell of claim 1 for the production of shinorine and/or porphyra-334 and/or mycosporine-glycine alanine, further comprising a fourth recombinant gene encoding a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 12 (NpF5597), 52 (Tery_2970), 74 (MysD [*Cyanobacterium stanieri* HL-69]), 76 (MysD [*Nostoc verrucosum* KU005]).

3. The genetically modified cyanobacterial cell of claim 1 for the production of mycosporine-2-glycine, further comprising a fifth recombinant gene encoding a fifth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to SEQ ID NO: 15 (Ap_MysD) or having at least 70%, preferably at least 80%,

most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Tery_2971).

4. A genetically modified cyanobacterial cell for the production of a mycosporine-like amino acid precursor 4-deoxygadusol, comprising

- a fusion recombinant gene encoding a fusion protein including first and second enzymes involved in the production of a mycosporine-like amino acid (MAA) in one polypeptide chain, the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity, preferably at least 90 % sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant), the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 MysB or MysB_HL-69 variant),

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant), and

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 MysB or MysB_HL-69 variant).

5. The genetically modified cyanobacterial cell of claim 4 for the production of mycosporine-glycine, further comprising at least one third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%,

preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 or MysC_HL-69 variant).

6. The genetically modified cyanobacterial cell of claim 5 for the production of shinorine, porphyra-334 and/or mycosporine-glycine-alanine, additionally including a fourth recombinant gene coding for a fourth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 12, 52, 74, or 76 (Tery-2970 and its homologues for production of shinorine**).**

7. The genetically modified cyanobacterial cell of claim 5 for the production of mycosporine-2-glycine, additionally including a fifth recombinant gene coding for a fifth enzyme which is at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 15 (Ap_MysD) or 50 (Tery_2971).

8**.** The genetically modified cyanobacterial cell of any of the claims 4 to 7, wherein the fusion recombinant gene encodes a fusion protein including a linker with between 18 to 24 amino acids, preferably wherein the linker is a linker selected from a group consisting of the amino acid sequences of SEQ ID NO: 82 or 84.

9. A genetically modified host cell for the production of a mycosporine-like amino acid Tery-347, comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant)

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 80% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase),

- a further recombinant gene encoding a further enzyme, wherein the further gene is selected from a group consisting of the following genes:

a) a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 or 50 (Ap_MysD or Tery_2971, MysD1),

b) a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% identical to either one of the SEQ ID NO: 12, 52, 74, 76 (NpF5597, Tery_2970, MysD_HL-69, Av_MysD).

10. A mycosporine-like amino acid (Tery-347.1), being a methylated porphyra-334 having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ

ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant),

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 80% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase), and

- a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 74 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 76 (NpF5597, MysD_HL-69 or Av_MysD).

11. The mycosporine-like amino acid (Tery-347.1) of claim 10, wherein Tery-347.1 has the molecular formula $C_{15}H_{24}N_2O_8$, preferably wherein its molecular weight is 360 Da.

12. A mycosporine-like amino acid (Tery-347.2), being a methylated shinorine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant),

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase), and

- a fourth gene encoding a fourth enzyme wherein the fourth gene is selected from a group consisting of: a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 52 (Tery_2970), and/or a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 74 (NpF5597 or MysD_HL-69).

13. The mycosporine-like amino acid (Tery-347.2) of claim 12, wherein Tery-347.2 has the molecular formula $C_{14}H_{22}N_2O_8$, preferably wherein its molecular weight is 346 Da.

14. A mycosporine-like amino acid (Tery-347.3), being a methylated mycosporine-2-glycine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB_HL-69 variant),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant),

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase), and

- a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Ap_MysD or Tery_2971, MysD1).

15. The mycosporine-like amino acid (Tery-347.3) of claim 14, wherein Tery-347.3 has the molecular formula $C_{13}H_{20}N_2O_7$, preferably wherein its molecular weight is 316 Da.

16. A genetically modified cyanobacterial host cell for the production of a mycosporine-like amino acid Tery-322, comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB of HL69),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant)

- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase).

17. The genetically modified cyanobacterial host cell of claim 16, wherein the sixth recombinant gene encodes a sixth enzyme being selected from a group consisting of: enzymes of SEQ ID NO: 90 (*Chondrocystis* sp. NIES-4102), SEQ ID NO: 88 (*Euhalothece* sp. KZN 001), SEQ ID NO: 86 (*Chroococcidiopsis* sp. TS-821) and SEQ ID NO: 92 (*Synechococcus* sp. PCC 7335 O-methyltransferase).

18. The genetically modified cyanobacterial host cell of claim 16 or 17 for the production of a mycosporine-like amino acid Tery-364, further comprising

- an eighth recombinant gene encoding an eighth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 56 (Tery_2968) or the eighth recombinant gene encoding an eighth enzyme

having at least 80%, preferably at least 90% sequence identity to SEQ ID NO: 104 (*Euhalothece* sp. KZN 001; homolog of Tery_2968).

19. The genetically modified cyanobacterial host cell of claim 18 wherein the eighth recombinant gene encodes an eighth enzyme being selected from a group consisting of: the enzyme of SEQ ID NO: 102 (*Scytonema cf. crispum* UCFS15), SEQ ID NO: 100 (Nostocales cyanobacterium HT-58-2), SEQ ID NO: 98 (*Cyanothece* sp. PCC 7424), SEQ ID NO: 96 (*Chlorogloeopsis fritschii*), SEQ ID NO: 94 (*Oscillatoria* sp. PCC 10802), SEQ ID NO: 104 (*Euhalothece* sp. KZN 001), and SEQ ID NO: 193 (NRPS of *Calothrix* sp. NIES-2100; homolog of Tery_2968).

20. A genetically modified cyanobacterial host cell for the production of a mycosporine-like amino acid palythine, comprising

- a first recombinant gene that encodes a first enzyme involved in the production of a mycosporine-like amino acid (MAA), the first enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (Ava-3858 MysA variant or distant clade MysA variant),

- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or the second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (Ava-3857 and MysB of HL69),

- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (Ava-3856 and MysC_HL-69 variant),

- a fifth gene encoding a fifth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 15 or having

at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 50 (Ap_MysD or Tery_2971, MysD1), and

- a seventh gene encoding a seventh enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with SEQ ID NO. 106 (*Calothrix sp. NIES*-2100**).**

21. The genetically modified cyanobacterial host cell of claim 20, wherein the seventh recombinant gene encodes a seventh enzyme being selected from a group consisting of: SEQ ID NO. 190 (*Scytonema tolypothrichoides* VB-61278) and SEQ ID NO. 106 (Calothrix sp. NIES-2100).

22. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of anyone of the above claims 1 to 21, wherein each of the recombinant genes is under the transcriptional control of a promoter which is an inducible promoter or a constitutive promoter.

23. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of the previous claim, wherein each of the promoters is inducible by a change of a metal-ion concentration, further wherein the promoter is preferably the PsmtA promoter or the PpetE promoter.

24. The genetically modified host cell of claim 9or the mycosporine-like amino acid of anyone of the claims 10 to 15, wherein the host cell is selected from a group consisting of bacteria and eukaryotic cells, preferably enterobacteria, such as *E. coli*, and more preferred cyanobacteria, such as *Cyanobacterium* sp., further wherein the cyanobacterial host cell is preferably *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4.

23. The genetically modified cyanobacterial cell of anyone of the above claims 1 to 8 or 16 to 21, wherein the cyanobacterial host cell is preferably *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp. ABCyano4, most preferably ABCyano4.

26. The genetically modified cyanobacterial cell of the previous claim, wherein the cyanobacterial host cell is *Cyanobacterium* sp. ABICyano1 or *Cyanobacterium* sp.

ABCyano4, having an extracellular capsular polysaccharide layer (CPS) with a thickness of between 1 and 3 μm, preferably between 1.1 and 2 μm.

27. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of anyone of the above claims 1 to 26, wherein any one of the recombinant gene is from a cyanobacterium.

28. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of anyone of the above claims 1 to 27, wherein the recombinant gene is codon optimized for improved expression in a cyanobacterial cell.

29. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of anyone of the above claims 1 to 28, wherein at least one recombinant gene is located on a modified endogenous or a heterologous extrachromosomal plasmid.

30. The genetically modified host cell, cyanobacterial cell or the mycosporine-like amino acid of anyone of the above claims 1 to 29, wherein the recombinant genes are located on an extrachromosomal high copy plasmid being present in a cell in at least 10, preferably at least 20, most preferably at least 40 copies per cell.

31. A method of producing an MAA in cyanobacterial cells or a host cells, comprising:

a) growing the genetically modified cyanobacterial cells or host cells of any of the above claims 1 to 9 and 16 to 30 in a culture medium; and

b) isolating the MAA from the cells and/or the culture medium.

32. The method of the above claim, wherein in step a) genetically modified cyanobacterial cells are grown with light and CO_2 addition.

33. The method of any of the claims 31 or 32, wherein step a) further comprises adding an amino acid and/or inducing the cells by adding an inducer to the culture medium.

34. The method of anyone of the above claims 31 to 33, wherein at least 50%, preferably at least 60% more preferred at least 70% of the MAA produced is associated with the cyanobacterial cell and wherein in step b) the MAA is isolated from the cells by separating the cells from the culture medium and isolating the MAA from the cells.

35. The method of the previous claim 34, wherein the cyanobacterial cell has a capsular exopolysaccharide layer (CPS) and wherein the MAA associated with the cell is associated with the CPS of the cyanobacterial cells.

36. The method of the previous claim 35, wherein in step b) the CPS is removed from the cells, thereby removing the MAA from the cells

37. A pharmaceutical composition or a cosmetic composition comprising a UV absorbing compound, wherein the UV absorbing compound is an MAA that has been produced in a genetically modified cyanobacterial cell or host cell of any of the above claims 1 to 9 and 16 to 30.

38. A mycosporine-like amino acid (Tery-347.4), being a methylated mycosporine-glycine-alanine and having an absorption maximum at 347 nm, being producible by culturing a genetically modified host cell, the genetically modified host cell comprising:

- a first recombinant gene that encodes the first enzyme involved in the production of a mycosporine-like amino acid, the first enzyme having at least 80% sequence identity, preferably at least 90% sequence identity with either the enzyme of SEQ ID NO: 3 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 68 (MysA),
- a second recombinant gene encoding a second enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 6 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 70 (mysB),
- a third recombinant gene encoding a third enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 9 or having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 72 (MysC),
- a sixth recombinant gene encoding a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 60 (Tery_2966) or the sixth recombinant gene encoding

a sixth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity to SEQ ID NO: 92 (O-methyltransferase from Synechococcus sp. PCC 7335), and

- a fourth gene encoding a fourth enzyme having at least 70%, preferably at least 80%, most preferably at least 90% sequence identity with the enzyme of SEQ ID NO: 12 (NpF5597 or Np_MysD).

39. The mycosporine-like amino acid (Tery-347.4) of claim 38, having the molecular formula $C_{14}H_{22}N_2O_7$, preferably wherein its molecular weight is 330 Da.

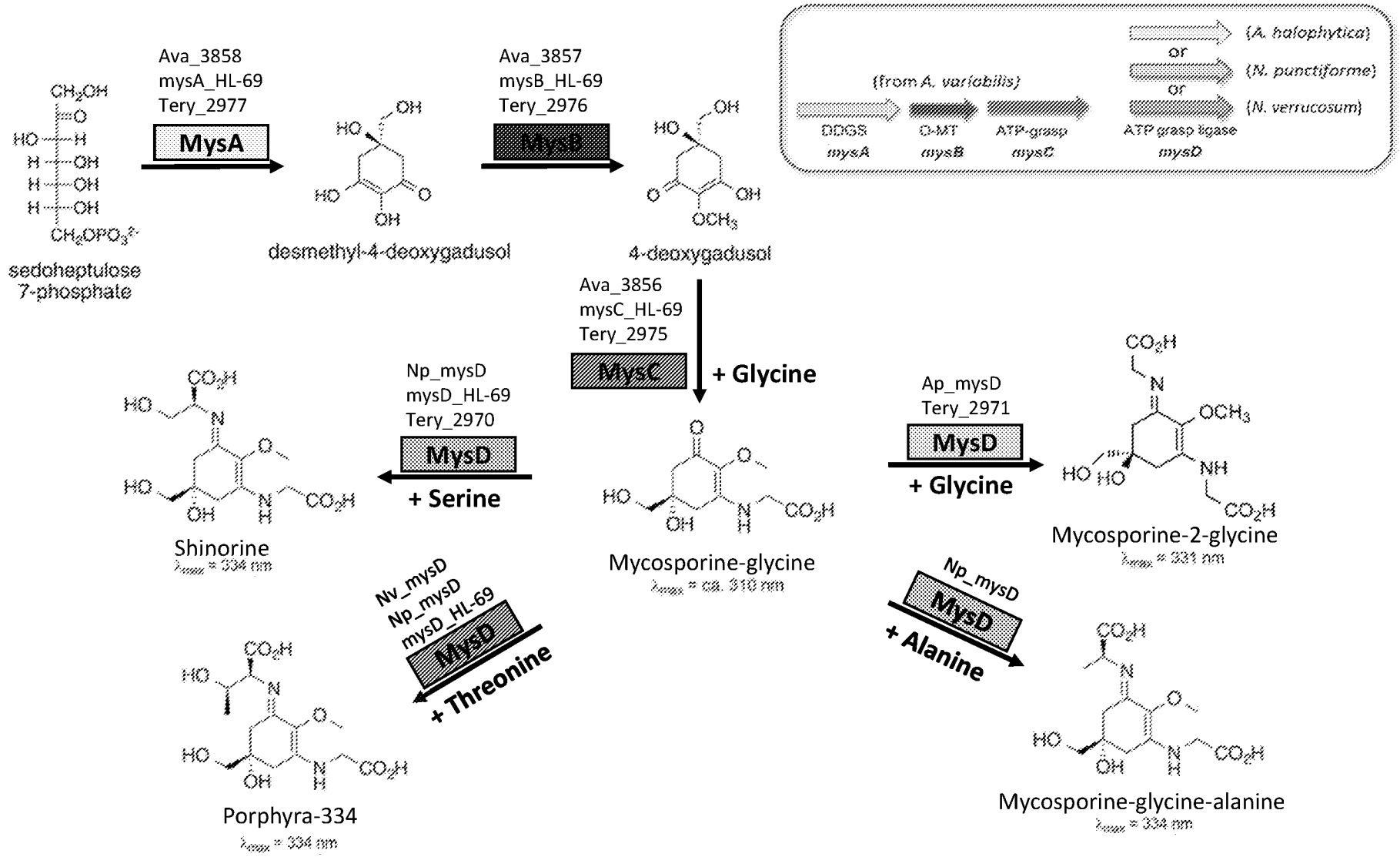

Ava_3858
mysA_HL-69
Tery_2977
MysA
Ava_3857
mysB_HL-69
Tery_2976
MysB
(from A. variabilis)
(A. halophytica)
or
(N. punctiforme)
or
(N. verrucosum)
ORF3
mysA
O-MT
mysB
ATP-grasp
mysC
ATP grasp ligase
mysD
sedoheptulose 7-phosphate
desmethyl-4-deoxygadusol
4-deoxygadusol
Ava_3856
mysC_HL-69
Tery_2975
MysC
+ Glycine
Np_mysD
mysD_HL-69
Tery_2970
MysD
+ Serine
Ap_mysD
Tery_2971
MysD
+ Glycine
Shinorine
Mycosporine-glycine
Mycosporine-2-glycine
Nv_mysD
Np_mysD
mysD_HL-69
MysD
+ Threonine
Np_mysD
MysD
+ Alanine
Porphyra-334
Mycosporine-glycine-alanine

FIG. 1

FIG. 2A

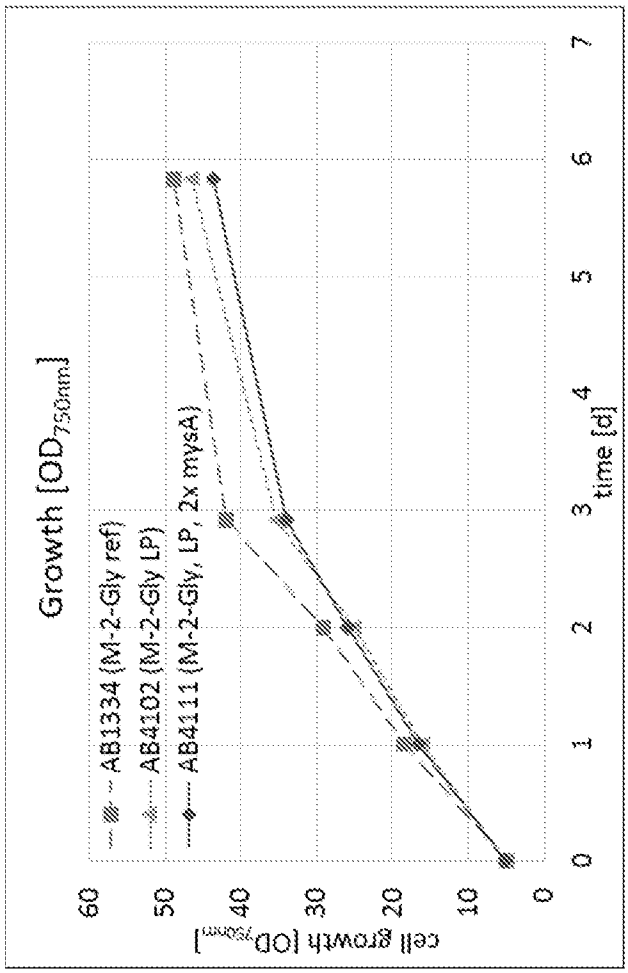

Growth [OD750nm]
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly LP)
AB4111 (M-2-Gly, LP, 2x mysA)
cell growth [OD750nm]
time [d]
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7

FIG. 2B

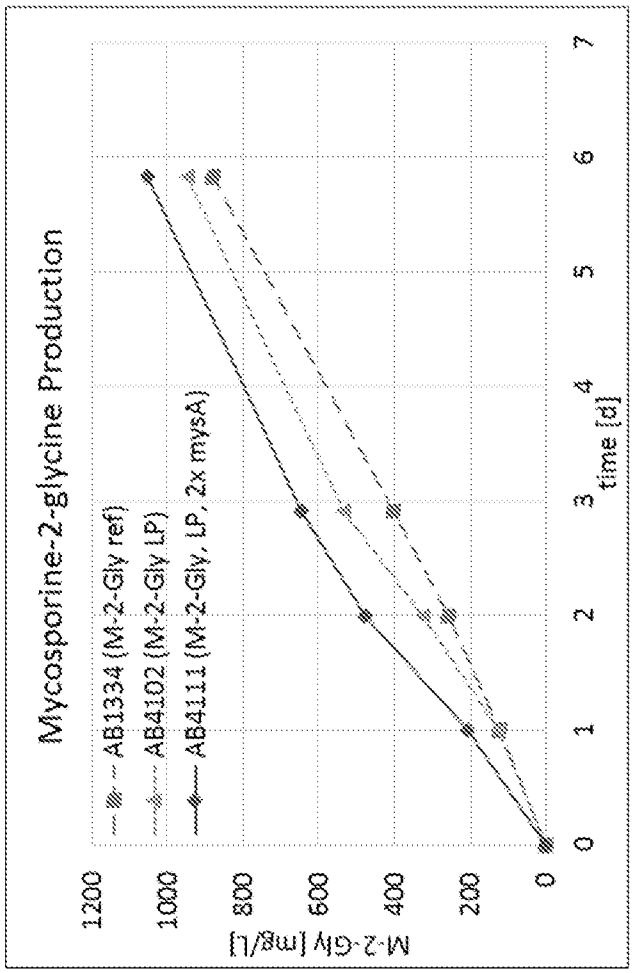

Mycosporine-2-glycine Production
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly LP)
AB4111 (M-2-Gly, LP, 2x mysA)
M-2-Gly [mg/L]
time [d]
0
200
400
600
800
1000
1200
0
1
2
3
4
5
6
7

FIG. 2C

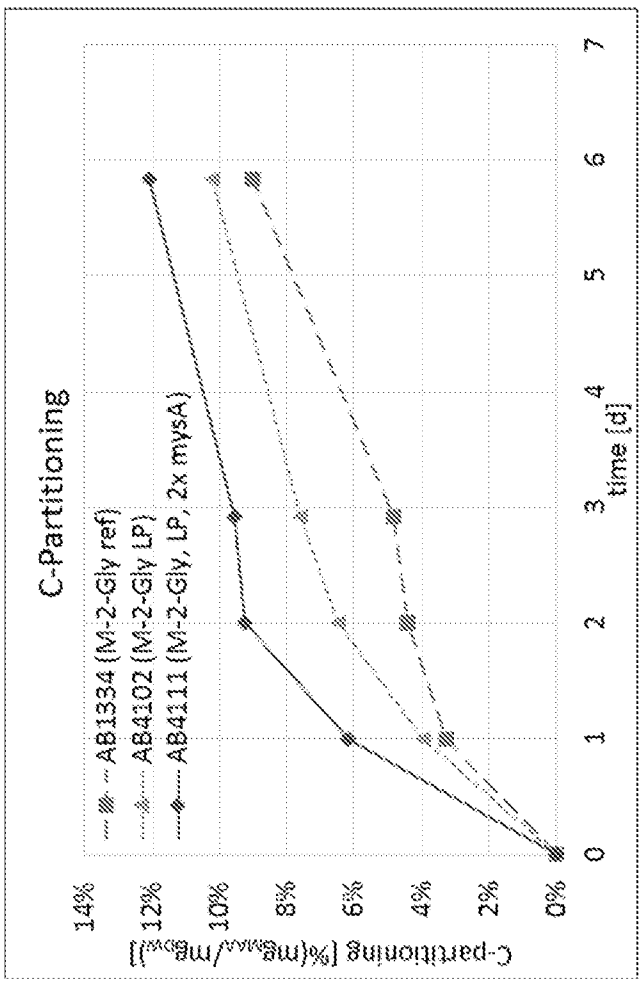

C-Partitioning
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly LP)
AB4111 (M-2-Gly, LP, 2x mysA)
C-partitioning [%(me_aaa/me_ex)]
14%
12%
10%
8%
6%
4%
2%
0%
0
1
2
3
4
5
6
7
time [d]

FIG. 3A

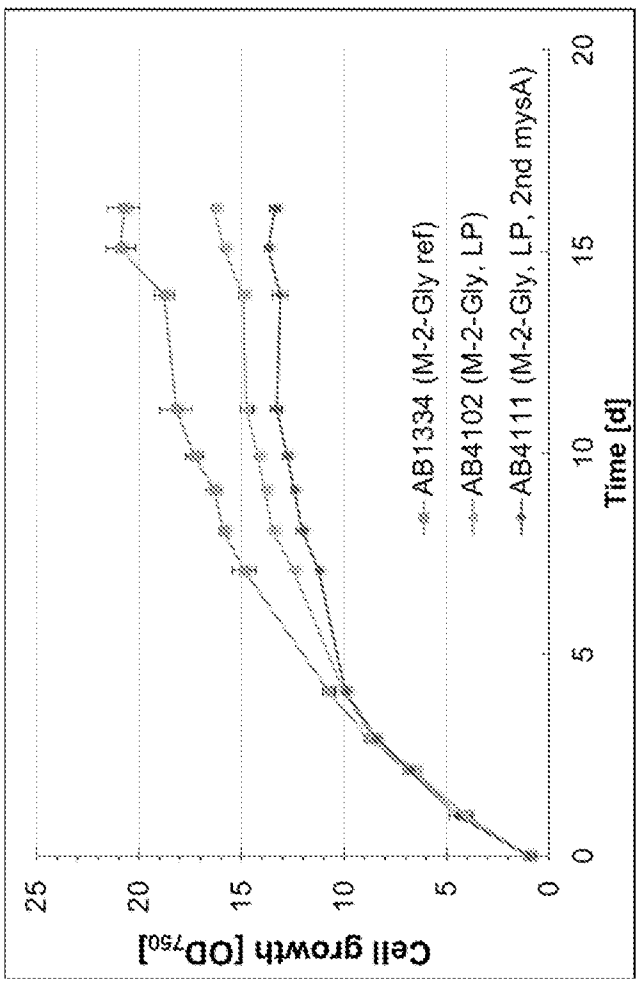

Cell growth [OD750]
25
20
15
10
5
0
0
5
10
15
20
Time [d]
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly, LP)
AB4111 (M-2-Gly, LP, 2nd mysA)

FIG. 3B

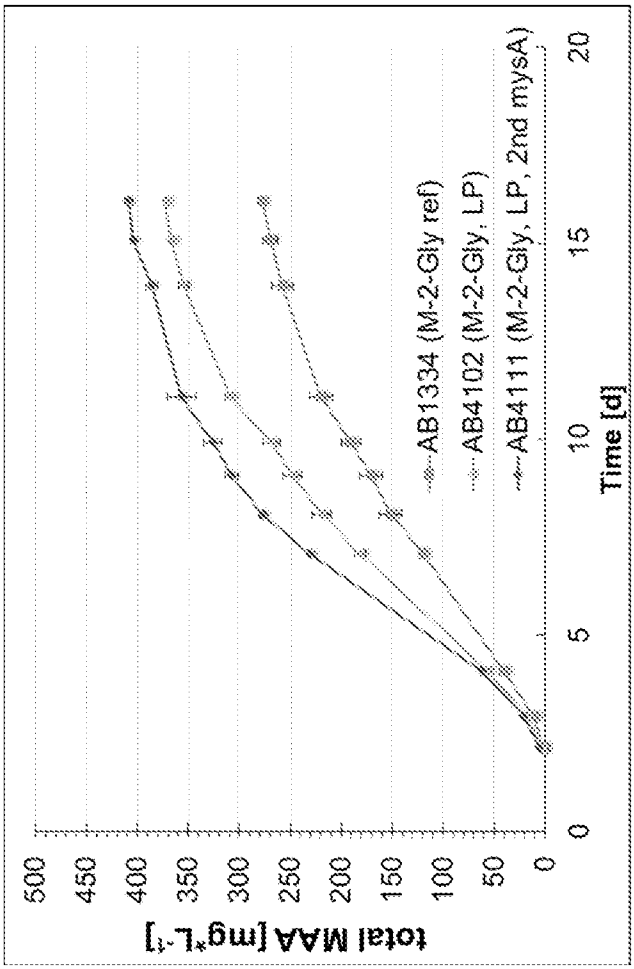

total MAA [mg*L-1]
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
Time [d]
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly, LP)
AB4111 (M-2-Gly, LP, 2nd mysA)

FIG. 3C

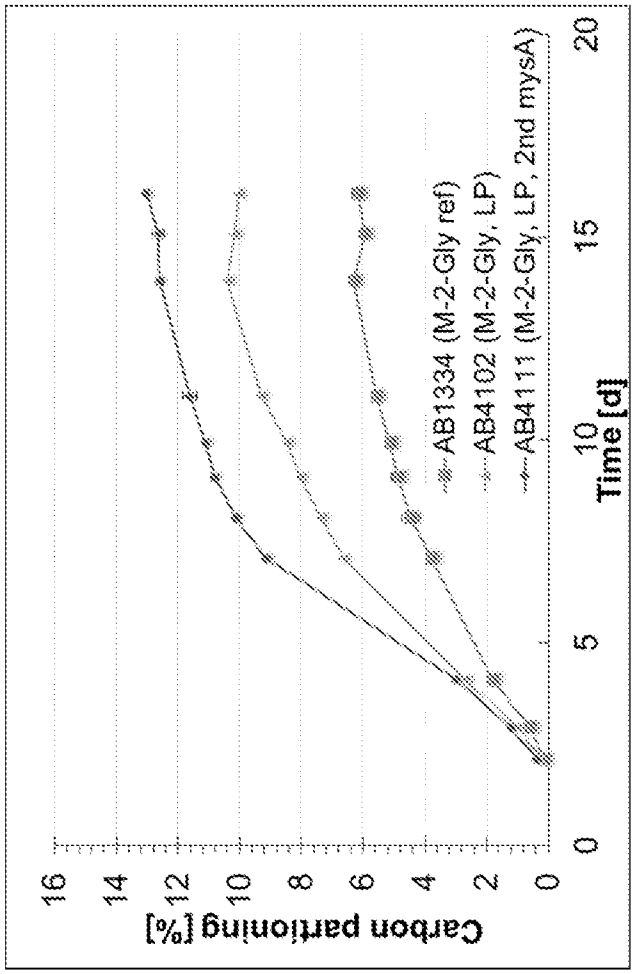

Carbon partioning [%]
16
14
12
10
8
6
4
2
0
0
5
10
15
20
Time [d]
AB1334 (M-2-Gly ref)
AB4102 (M-2-Gly, LP)
AB4111 (M-2-Gly, LP, 2nd mysA)

FIG. 3D

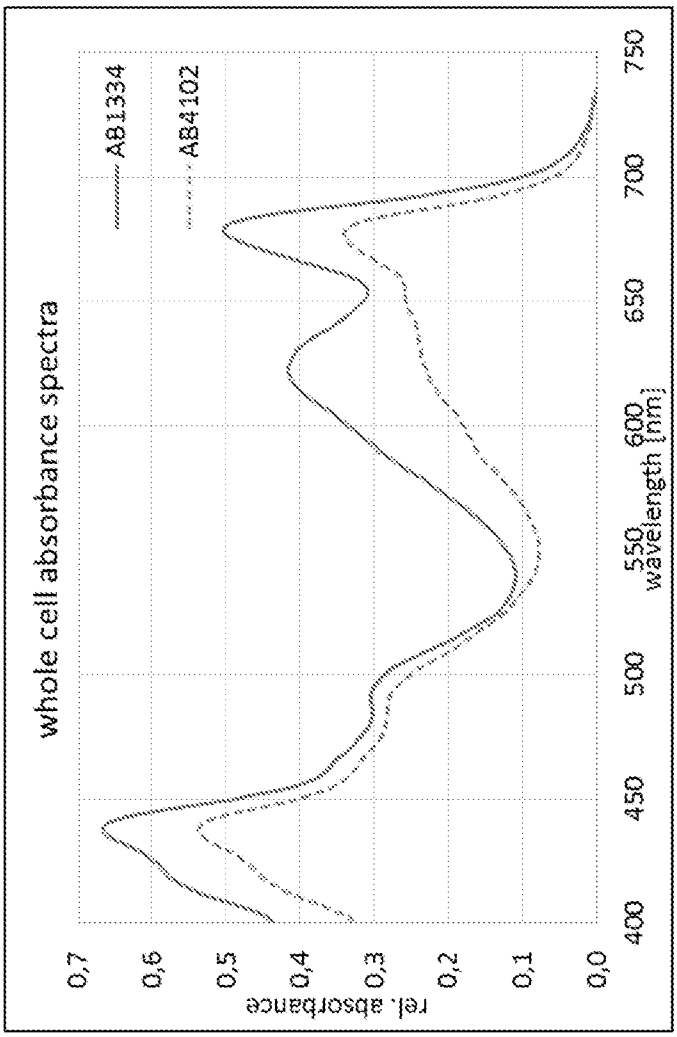

whole cell absorbance spectra
AB1334
AB4102
rel. absorbance
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
400
450
500
550
600
650
700
750
wavelength [nm]

FIG. 4A

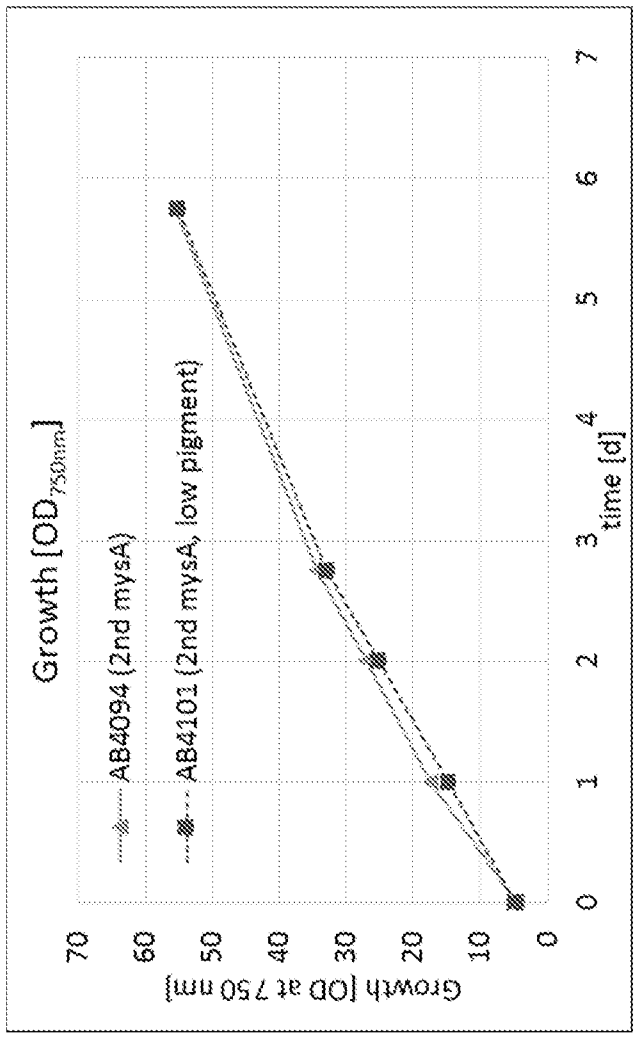

Growth [OD750nm]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)
Growth [OD at 750 nm]
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6
7
time [d]

FIG. 4B

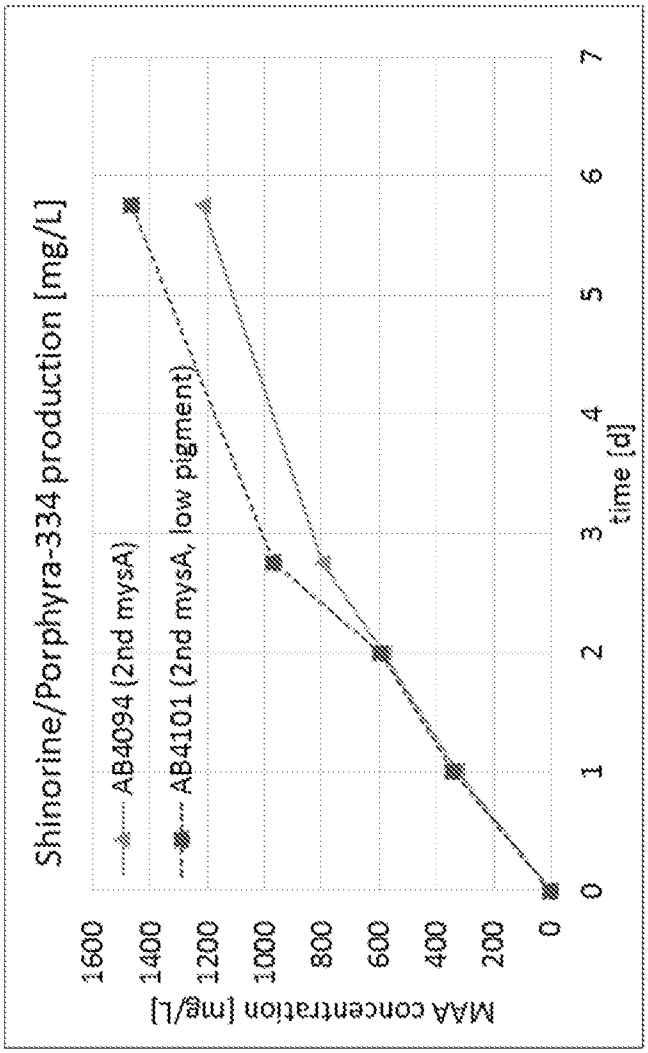

Shinorine/Porphyra-334 production [mg/L]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)
MAA concentration [mg/L]
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
time [d]

FIG. 4C

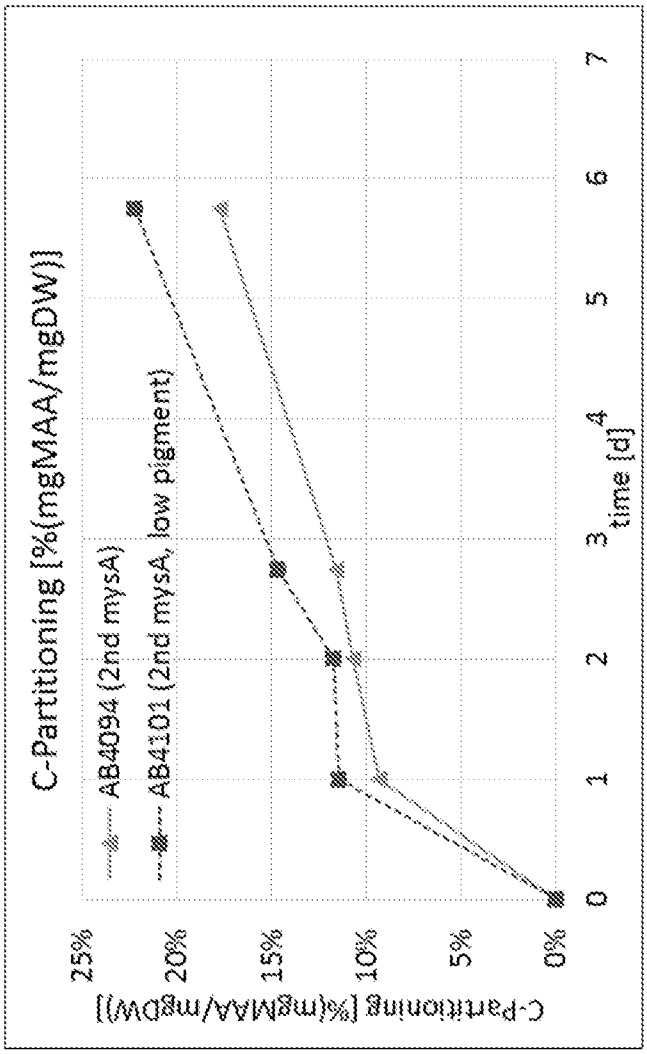

C-Partitioning [%(mgMAA/mgDW)]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)
C-Partitioning [%(mgMAA/mgDW)]
25%
20%
15%
10%
5%
0%
0
1
2
3
4
5
6
7
time [d]

FIG. 5A

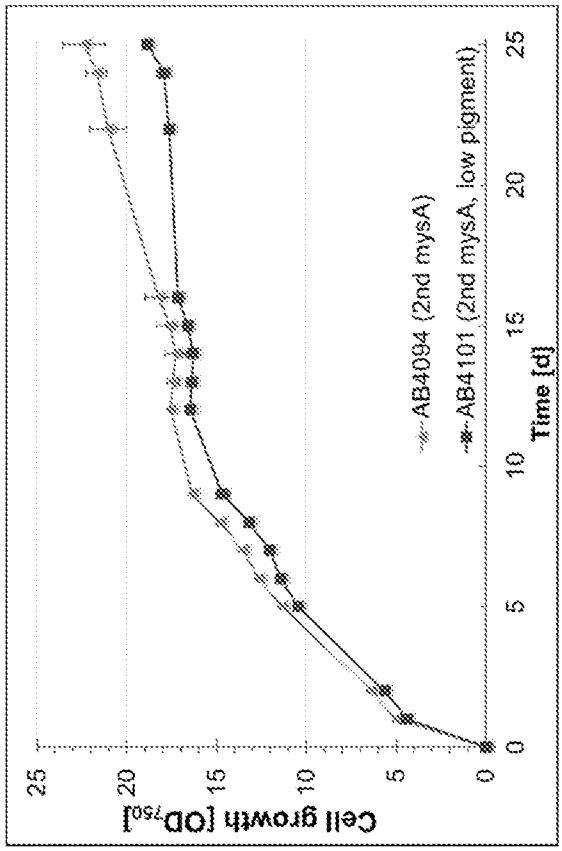

Cell growth [OD750]
Time [d]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)
0
5
10
15
20
25

FIG. 5B

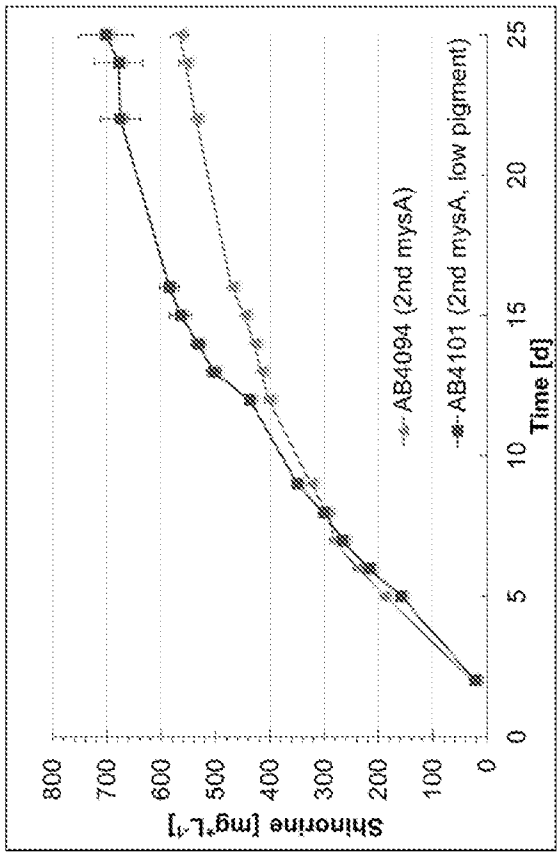

Shinorine [mg*L-1]
Time [d]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)
0
100
200
300
400
500
600
700
800
5
10
15
20
25

FIG. 5C

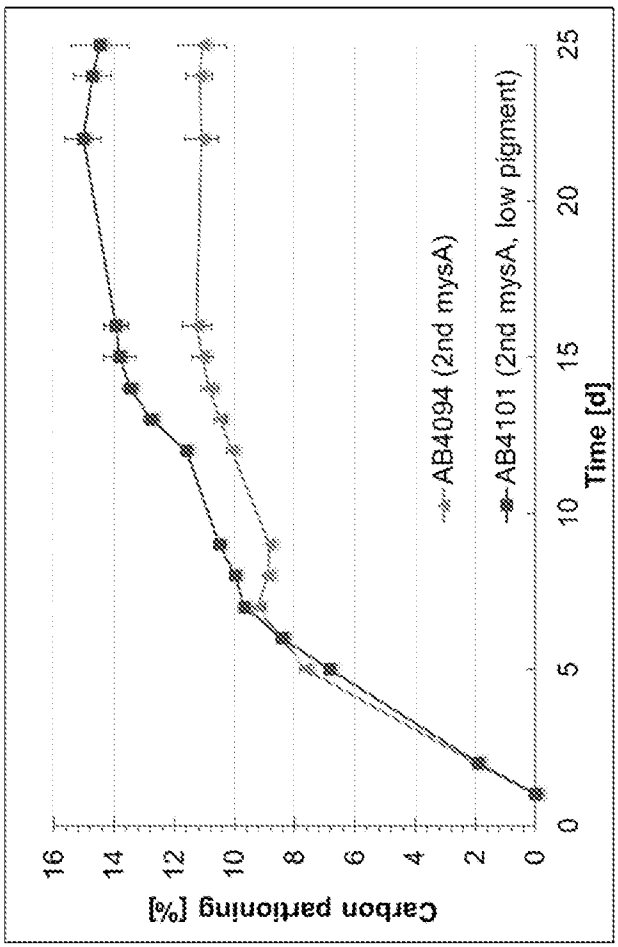

Carbon partioning [%]
0
2
4
6
8
10
12
14
16
0
5
10
15
20
25
Time [d]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)

FIG. 5D

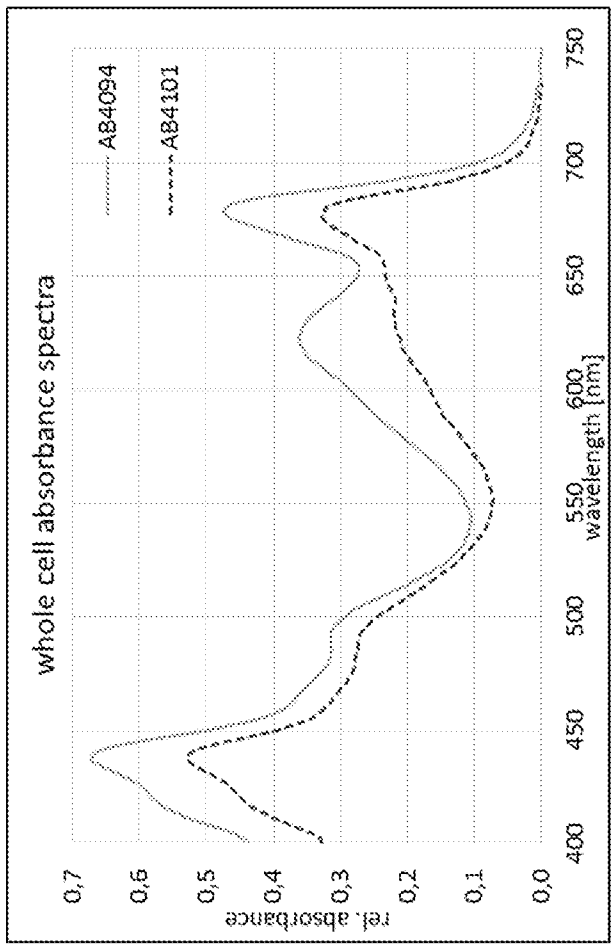

whole cell absorbance spectra
rel. absorbance
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
400
450
500
550
600
650
700
750
wavelength [nm]
AB4094
AB4101

FIG. 6A

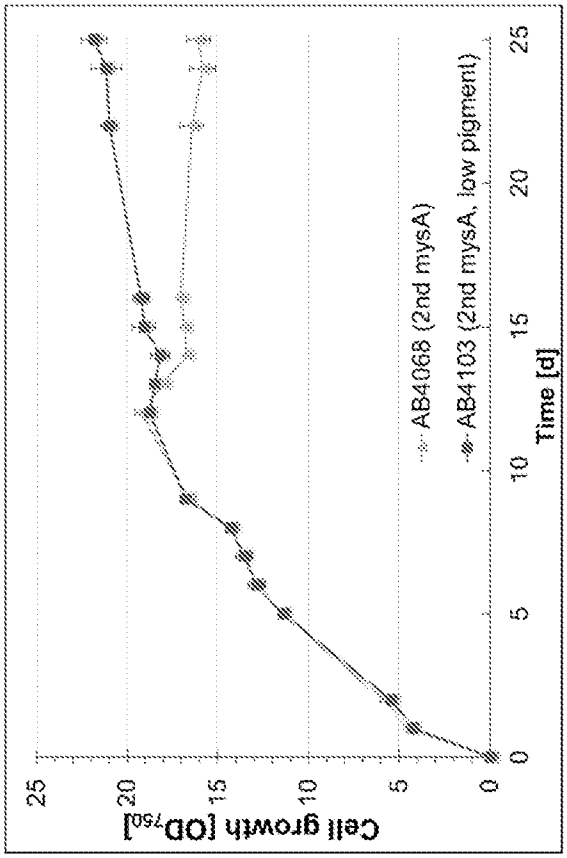

Cell growth [OD750]
Time [d]
AB4068 (2nd mysA)
AB4103 (2nd mysA, low pigment)
0
5
10
15
20
25

FIG. 6B

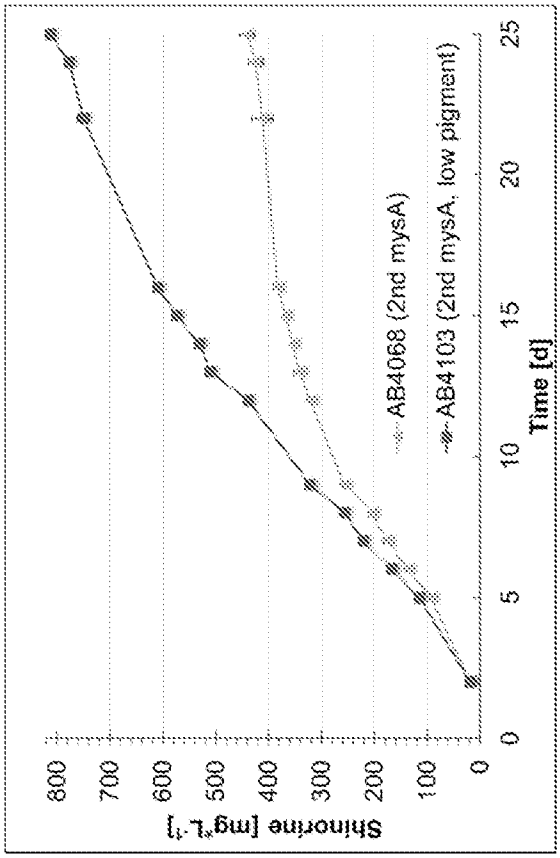

Shinorine [mg*L-1]
Time [d]
AB4068 (2nd mysA)
AB4103 (2nd mysA, low pigment)
0
100
200
300
400
500
600
700
800
0
5
10
15
20
25

FIG. 6C

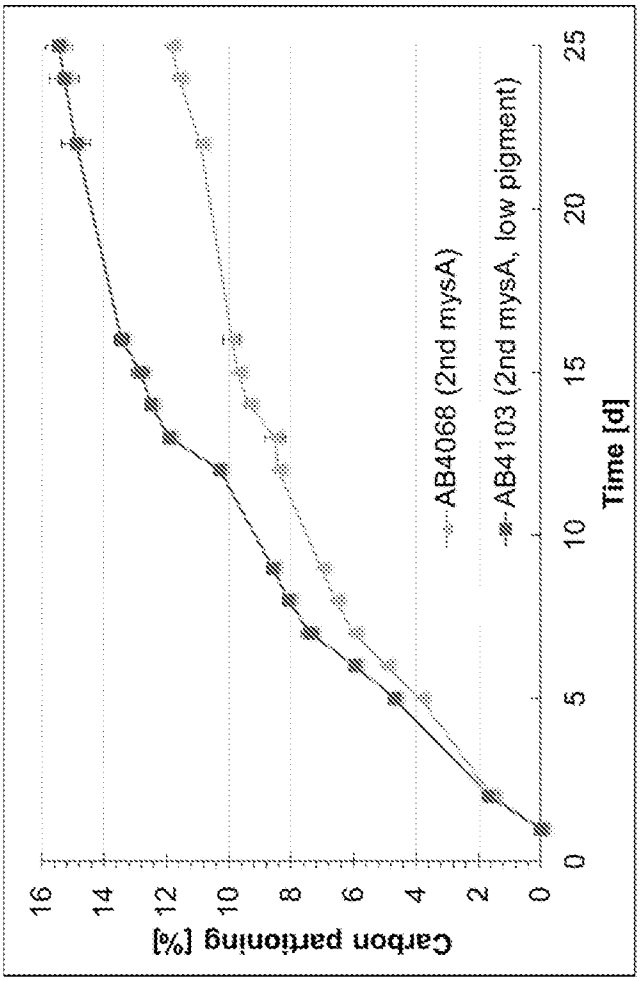
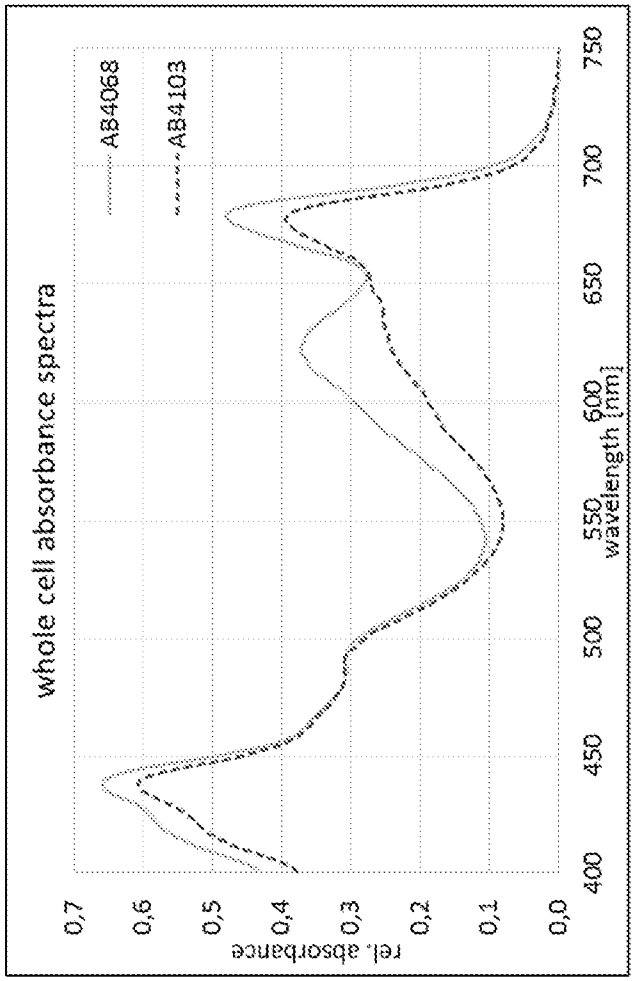

Carbon partioning [%]
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
Time [d]
AB4068 (2nd mysA)
AB4103 (2nd mysA, low pigment)

FIG. 6D


whole cell absorbance spectra
rel. absorbance
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
400
450
500
550
600
650
700
750
wavelength [nm]
AB4068
AB4103

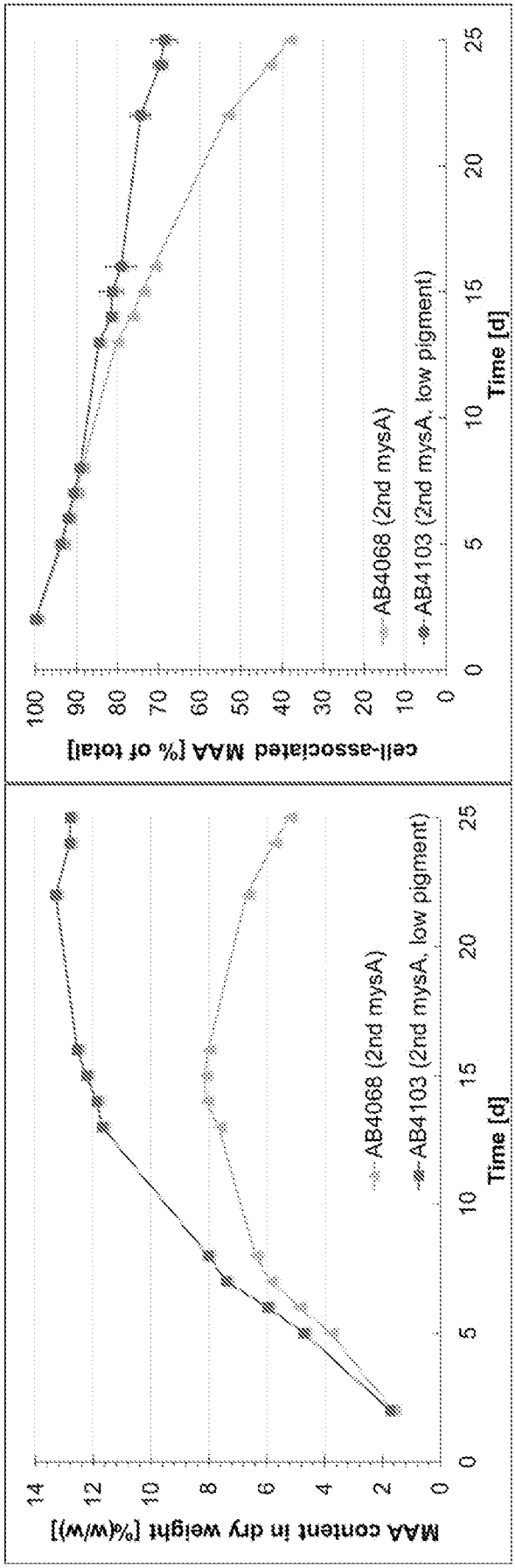

MAA content in dry weight [%(w/w)]
0
2
4
6
8
10
12
14
0
5
10
15
20
25
Time [d]
AB4068 (2nd mysA)
AB4103 (2nd mysA, low pigment)
cell-associated MAA [% of total]
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
Time [d]
AB4068 (2nd mysA)
AB4103 (2nd mysA, low pigment)

FIG. 7A

FIG. 7B

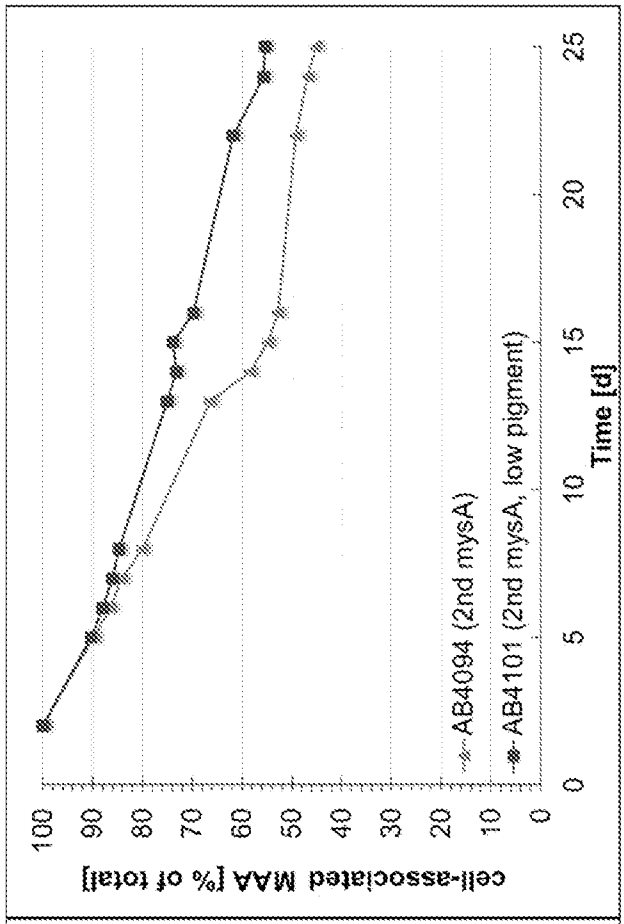

cell-associated MAA [% of total]
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
Time [d]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)

FIG. 7D

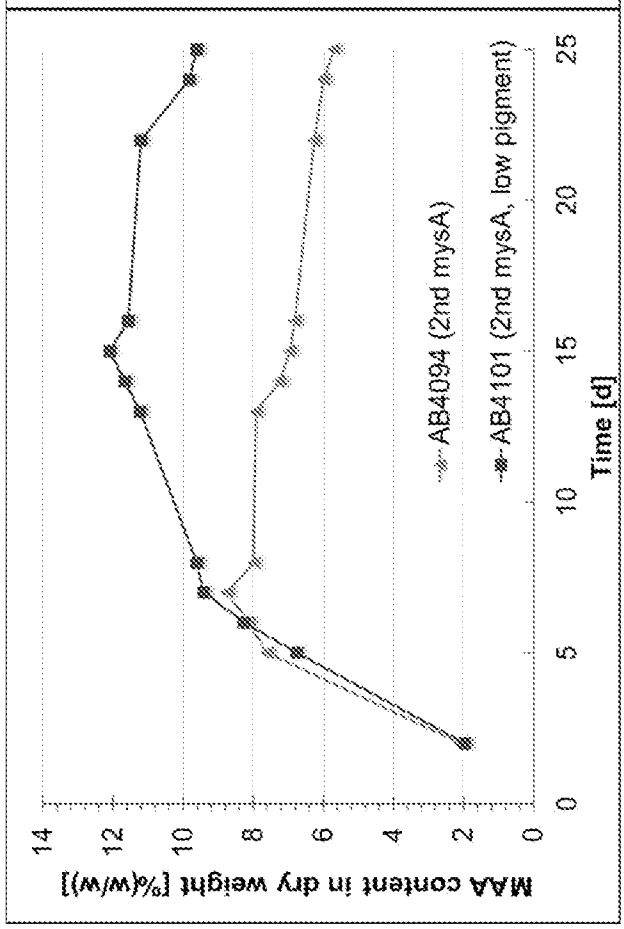

MAA content in dry weight [%(w/w)]
14
12
10
8
6
4
2
0
0
5
10
15
20
25
Time [d]
AB4094 (2nd mysA)
AB4101 (2nd mysA, low pigment)

FIG. 7C

AB4100	p1710B-PsmtA-**mysABC**-PsmtA-**mysD**	p1710C-PsmtA-**mysA**-PrbcL-**mys**D (control)	→ 2nd mysA, 2nd mysD
AB4179	p1710B-PsmtA-**mysABC**-PsmtA-**mysD**	p1710C-PsmtA-**mysA-linkerX-mysB**-PrbcL-**mysD**	→ mysAB fusion, 2nd mysD
AB4181	p1710B-PsmtA-**mysABC**-PsmtA-**mysD**	p1710C-PsmtA-**mysA-linkerY-mysB**-PrbcL-**mysD**	→ mysAB fusion, 2nd mysD

peptide linker X: GSSSGSS EAAAK EAAAK GSSSGSS
peptide linker Y: EAAAK GSSSGSS GSSSGSS EAAAK

FIG. 8A

- basal expression of native MysA, MysB, MysC and MysD

- In addition, expression of an artificial MysA-MysB fusion protein (2 linker variants)

linker X

linker Y

FIG. 8B

FIG. 9A

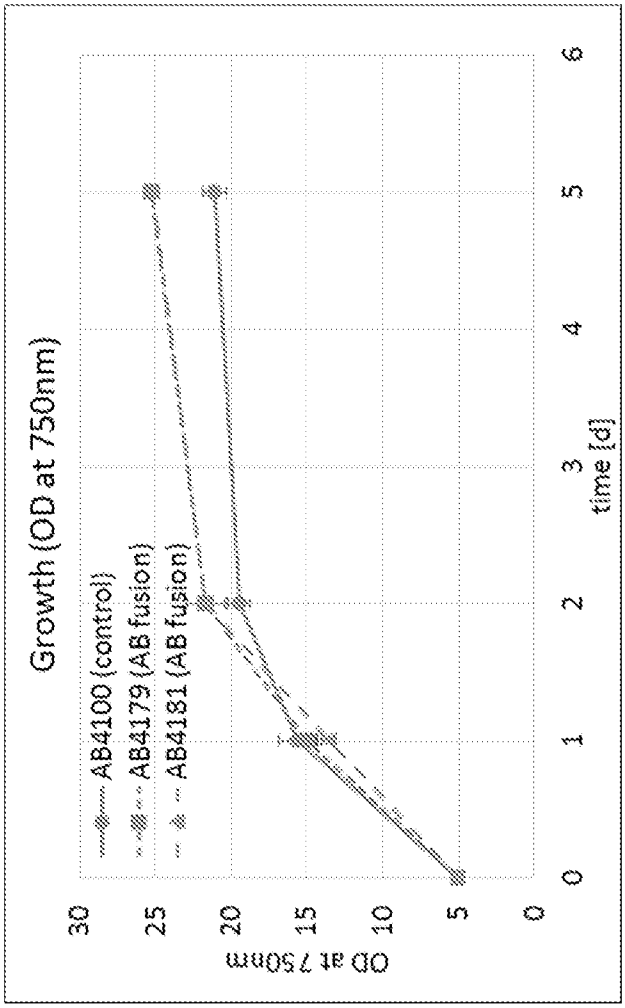
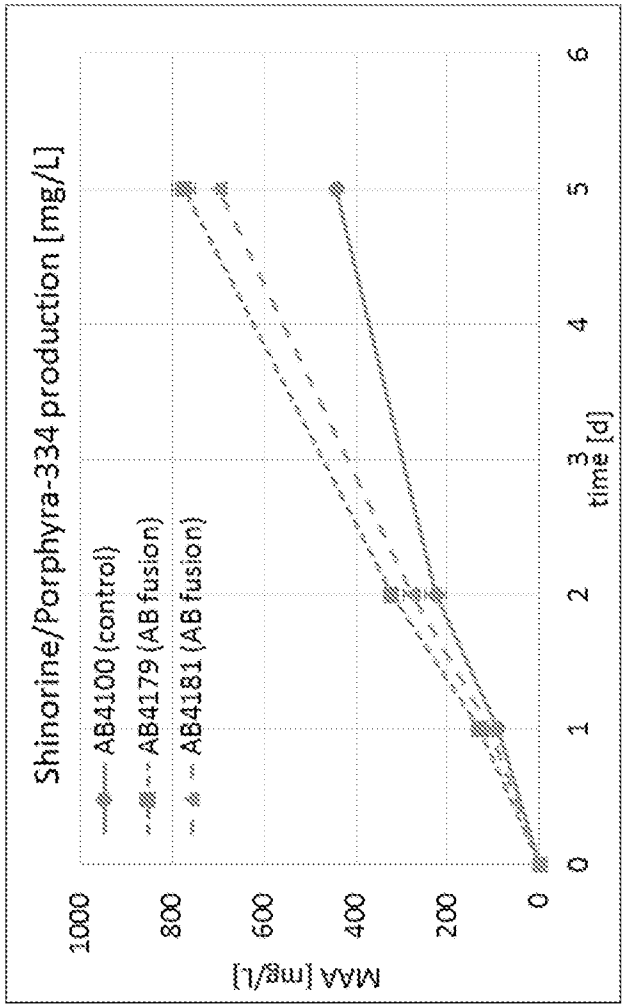

Growth (OD at 750nm)
AB4100 (control)
AB4179 (AB fusion)
AB4181 (AB fusion)
OD at 750nm
time [d]
0
5
10
15
20
25
30
0
1
2
3
4
5
6

FIG. 9B


Shinorine/Porphyra-334 production [mg/L]
AB4100 (control)
AB4179 (AB fusion)
AB4181 (AB fusion)
MAA [mg/L]
time [d]
0
200
400
600
800
1000
0
1
2
3
4
5
6

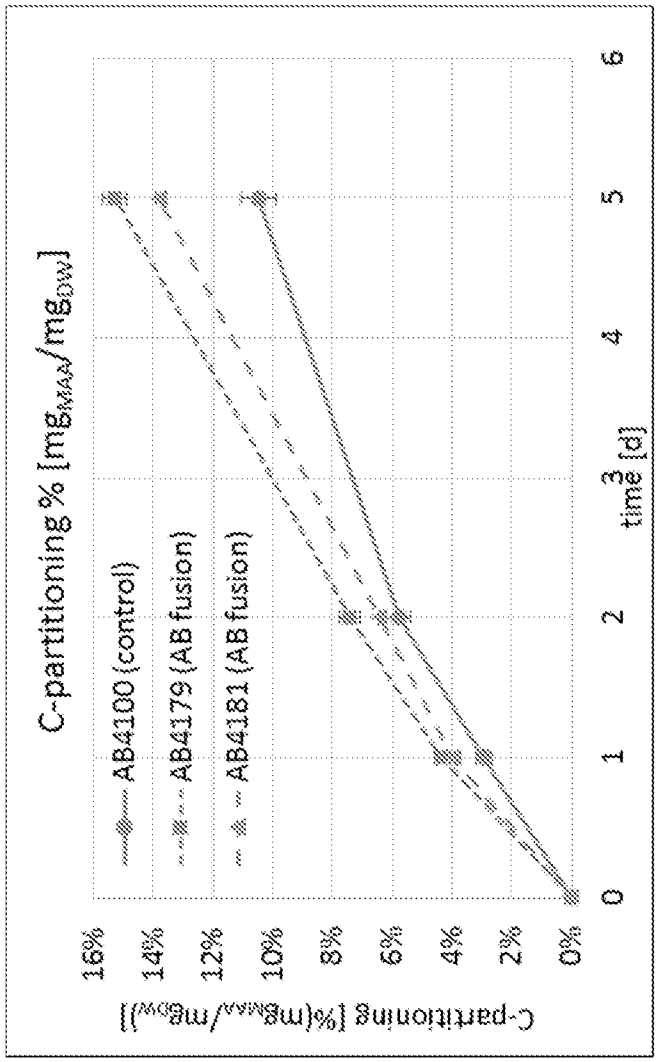

C-partitioning % [mg$_{MAA}$/mg$_{DW}$]
AB4100 (control)
AB4179 (AB fusion)
AB4181 (AB fusion)
C-partitioning [%(mg$_{MAA}$/mg$_{DW}$)]
16%
14%
12%
10%
8%
6%
4%
2%
0%
0
1
2
3
4
5
6
time [d]

FIG. 9C

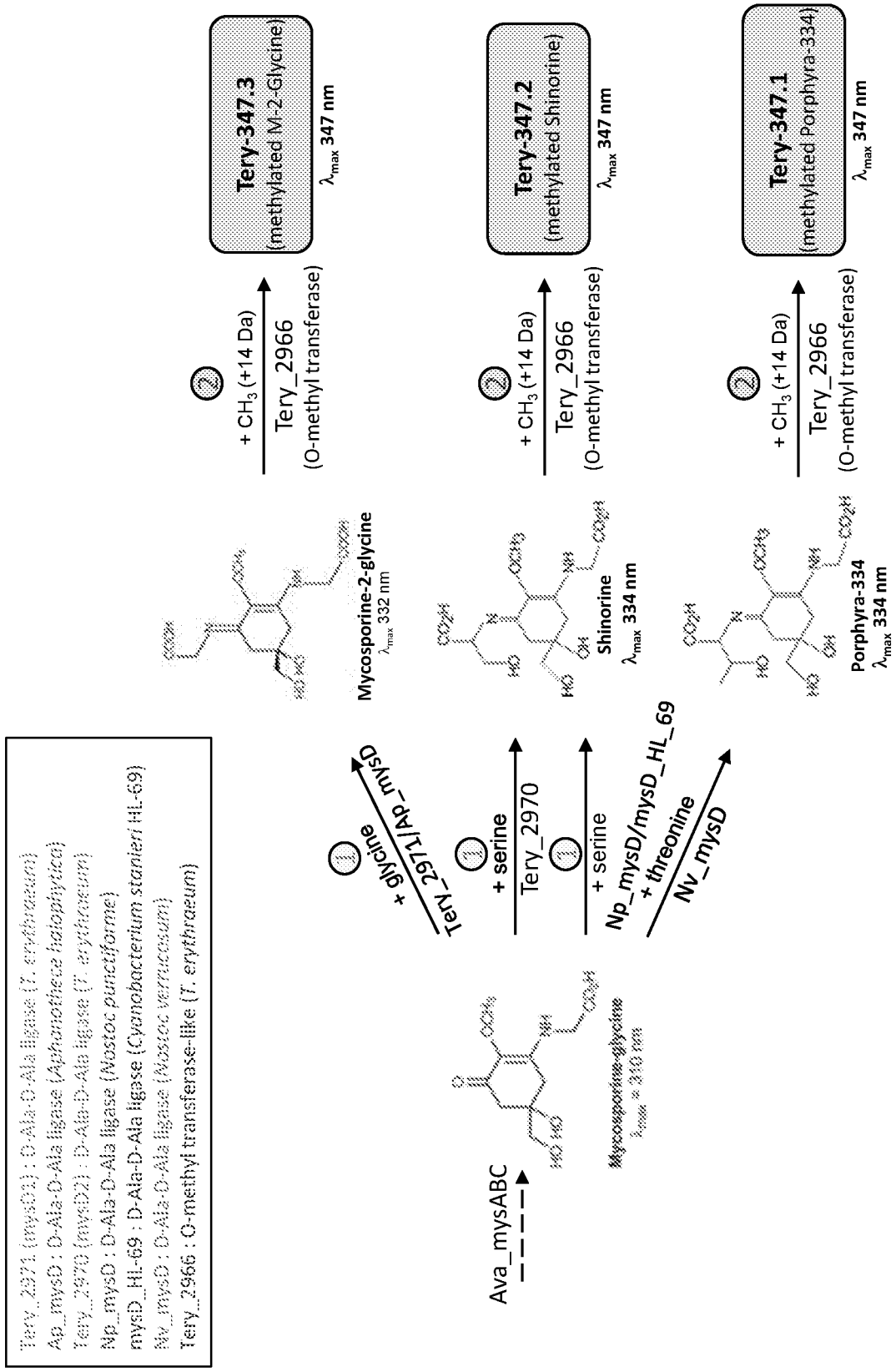
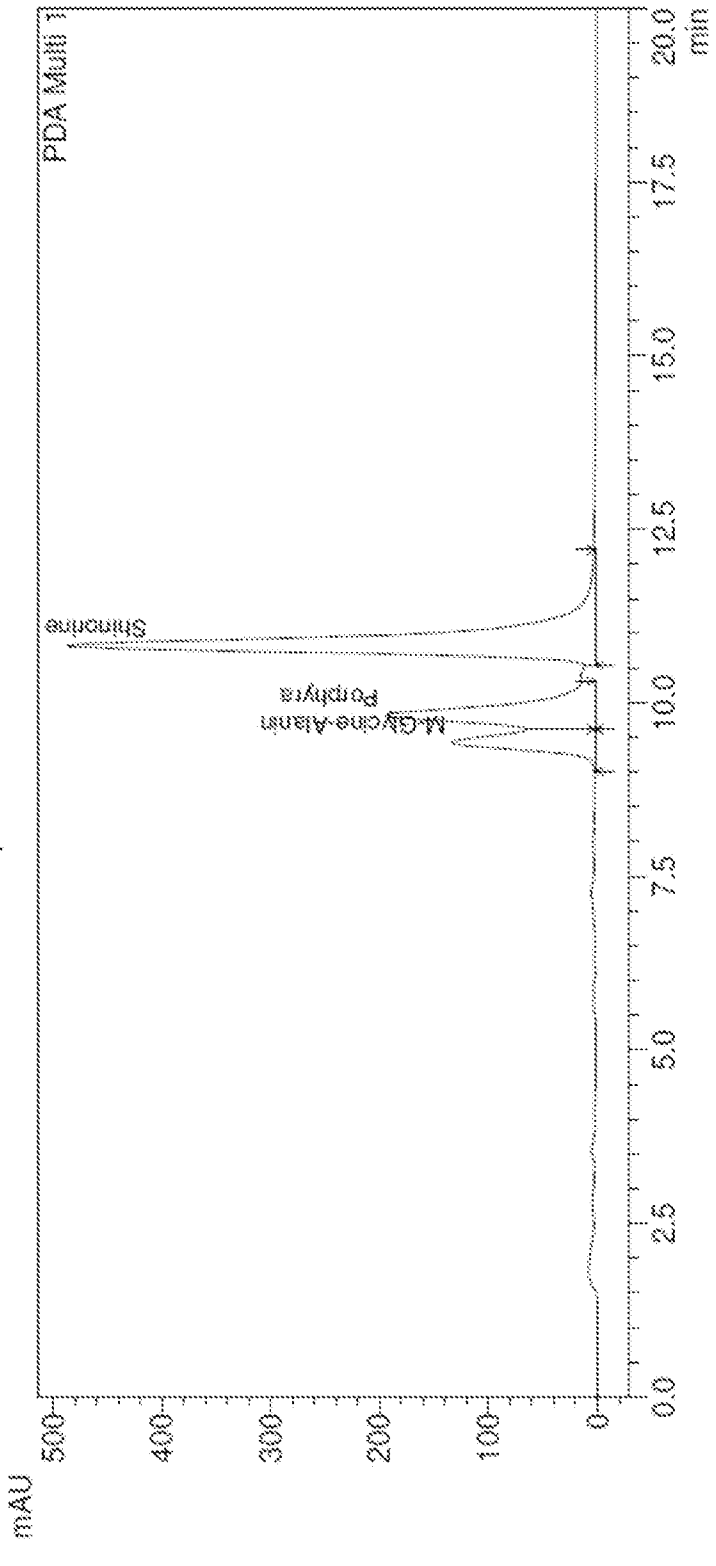

Tery_2971 (mysD1) : D-Ala-D-Ala ligase (T. erythraeum)
Ap_mysD : D-Ala-D-Ala ligase (Aphanothece halophytica)
Tery_2970 (mysD2) : D-Ala-D-Ala ligase (T. erythraeum)
Np_mysD : D-Ala-D-Ala ligase (Nostoc punctiforme)
mysD_HL-69 : D-Ala-D-Ala ligase (Cyanobacterium stanieri HL-69)
Nv_mysD : D-Ala-D-Ala ligase (Nostoc verrucosum)
Tery_2966 : O-methyl transferase-like (T. erythraeum)
Ava_mysABC
Mycosporine-glycine
λmax = 310 nm
① + glycine
Tery_2971/Ap_mysD
① + serine
Tery_2970
① + serine
Np_mysD/mysD_HL_69
+ threonine
Nv_mysD
Mycosporine-2-glycine
λmax 332 nm
Shinorine
λmax 334 nm
Porphyra-334
λmax 334 nm
② + CH3 (+14 Da)
Tery_2966
(O-methyl transferase)
Tery-347.3
(methylated M-2-Glycine)
λmax 347 nm
Tery-347.2
(methylated Shinorine)
λmax 347 nm
Tery-347.1
(methylated Porphyra-334)
λmax 347 nm

FIG. 10

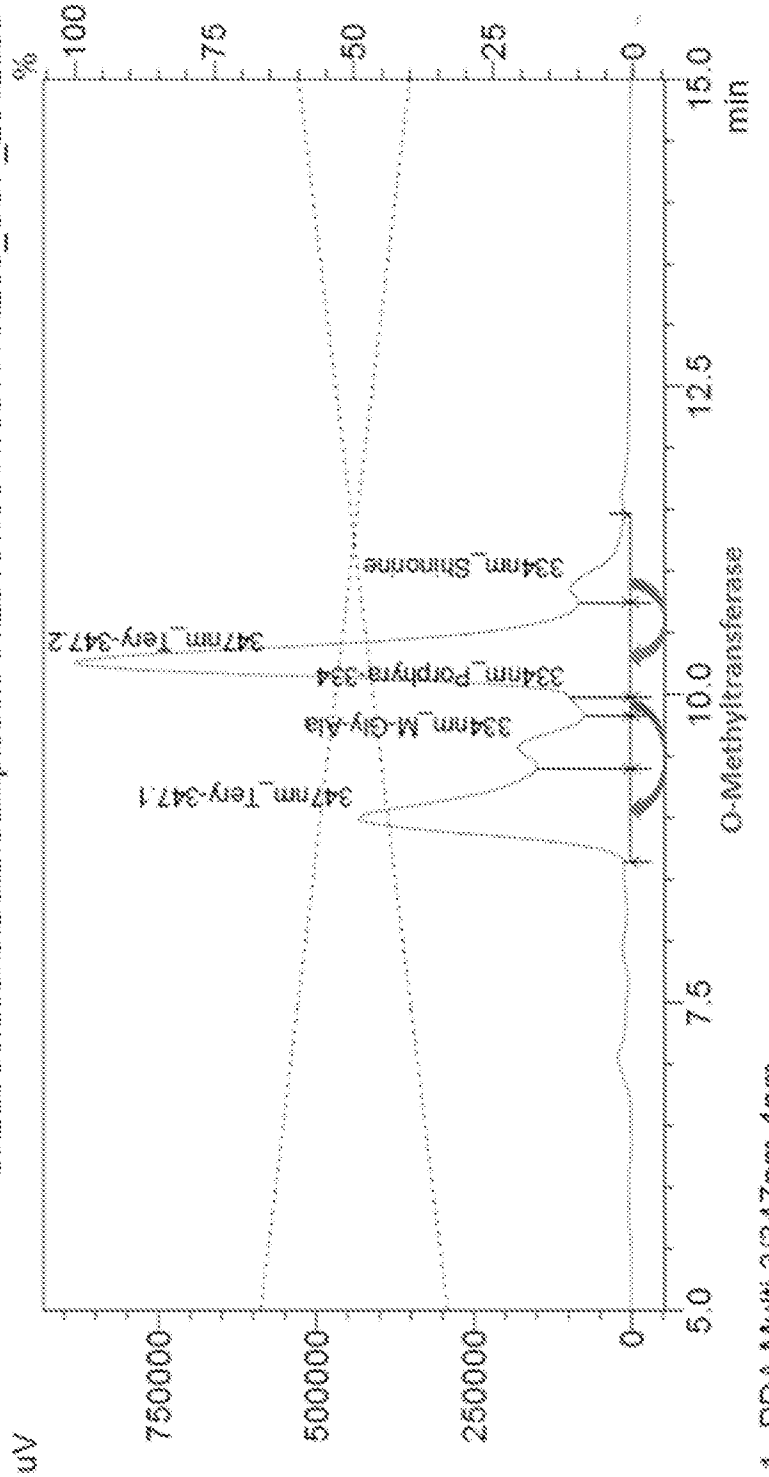


Peak Table

PDA Ch1 320nm 4nm

Name	Ret. Time	Area	Area %
M-Glycine-Alanin	9.423	2082665	15.1
Porphyra	9.813	3450566	25.0
Shinorine	10.815	8292906	59.9
		13826137	100.0

FIG. 11



PeakTable

PDA Ch3 347nm 4nm

Name	Ret. Time	Area	Area %
347nm_Tery-347.1	8.994	10342907	30.3
334nm_M-Gly-Ala	9.566	3548923	10.4
334nm_Porphyra-334	9.973	821742	2.4
347nm_Tery-347.2	10.236	17494976	51.2
334nm_Shinorine	10.860	1950578	5.7
		34156128	100.0

Porphyra → Tery-347.1 ~ 80%

Shinorine → Tery-347.2 ~ 90%

FIG. 12

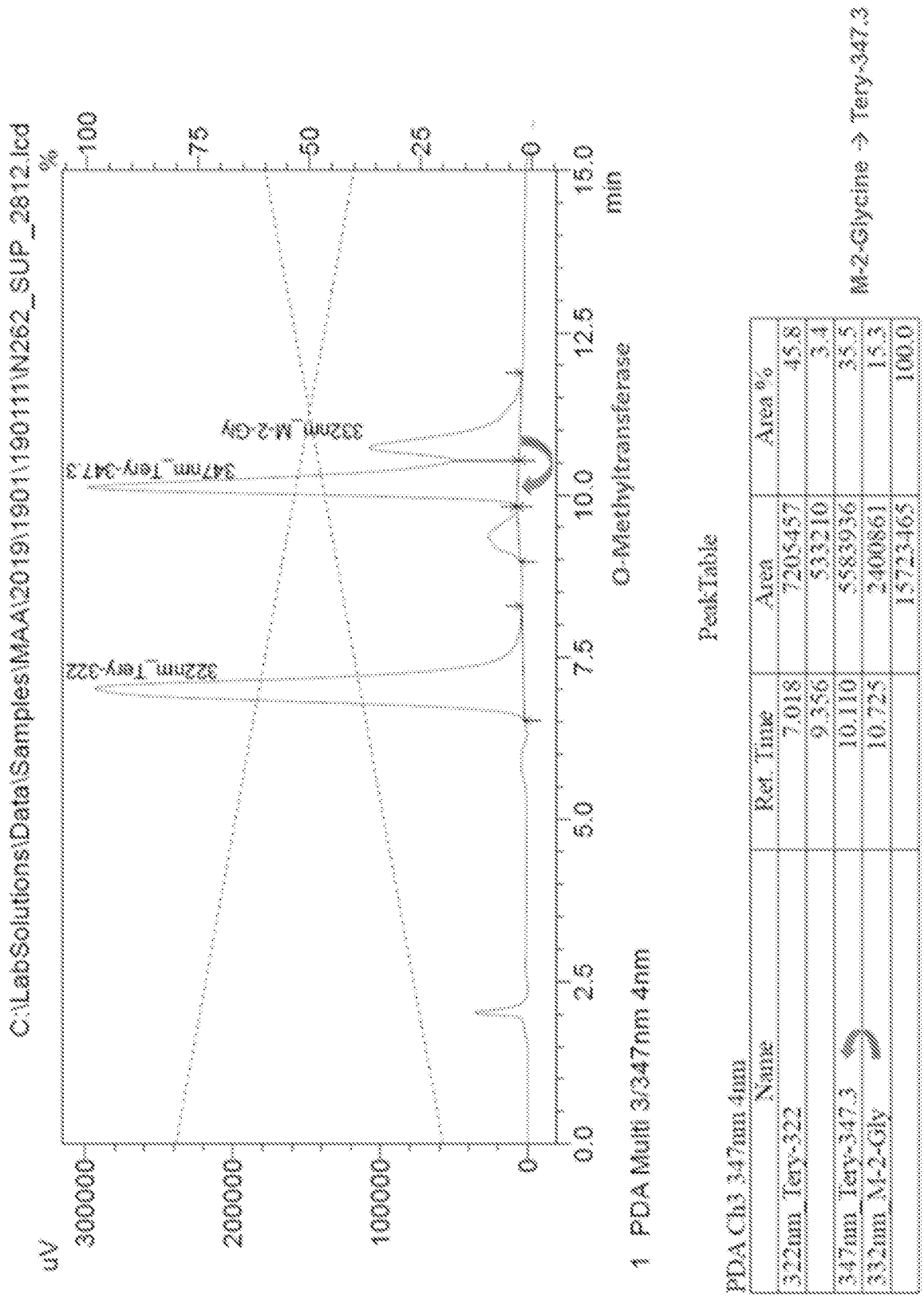

C:\LabSolutions\Data\Samples\MAA\2019\190111\190111N262_SUP_2812.lcd
uV
%
min
322nm_Tery-322
347nm_Tery-347.3
332nm_M-2-Gly
O-Methyltransferase
1 PDA Multi 3/347nm 4nm
PeakTable
PDA Ch3 347nm 4nm
Name
Ret. Time
Area
Area %
322nm Tery-322
7.018
7205437
45.8
9.356
533210
3.4
347nm Tery-347.3
10.110
5583936
35.5
332nm M-2-Gly
10.725
2400861
15.3
15723465
100.0
M-2-Glycine → Tery-347.3

FIG. 13A

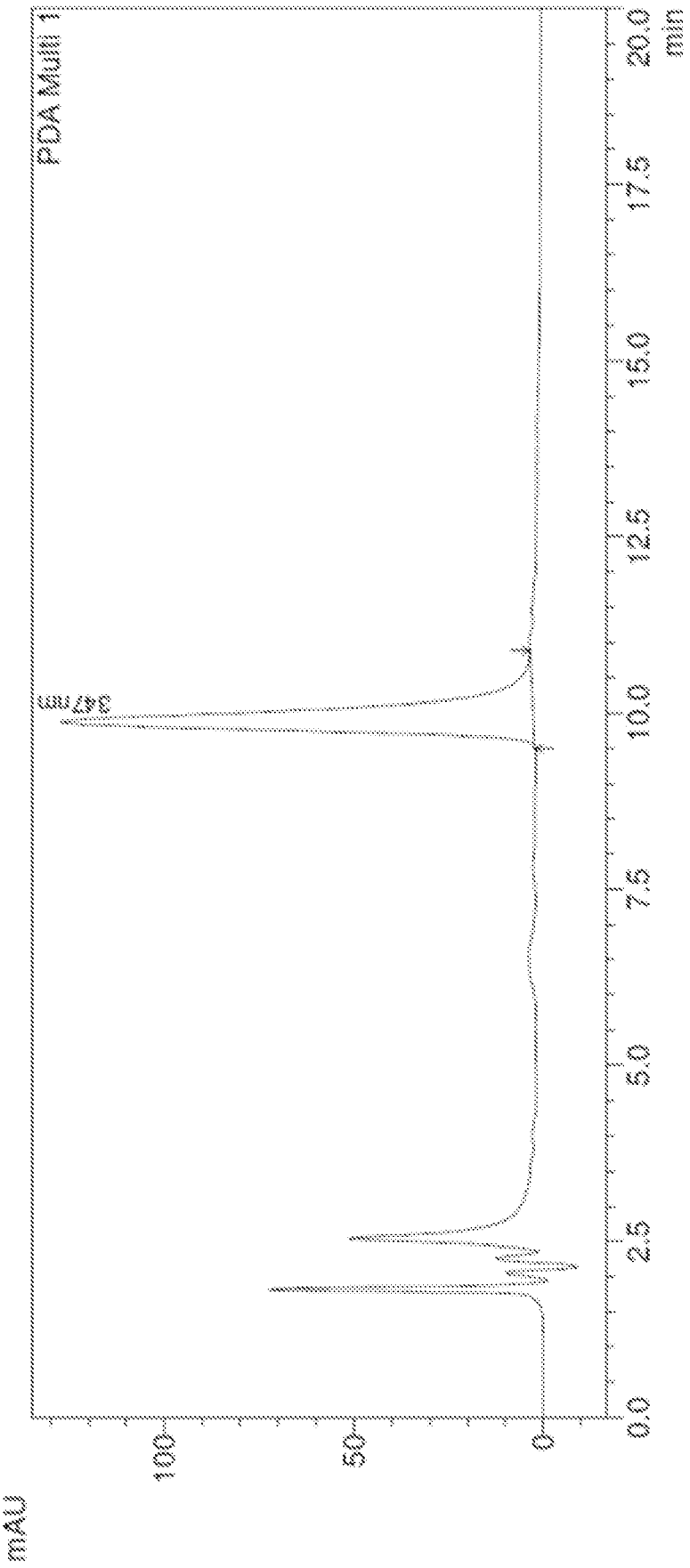

C:\LabSolutions\Data\Samples\MAA\2019\1901\190115\3186_17C.lcd
PDA Multi 1
347nm
mAU
100
50
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min

FIG. 13B

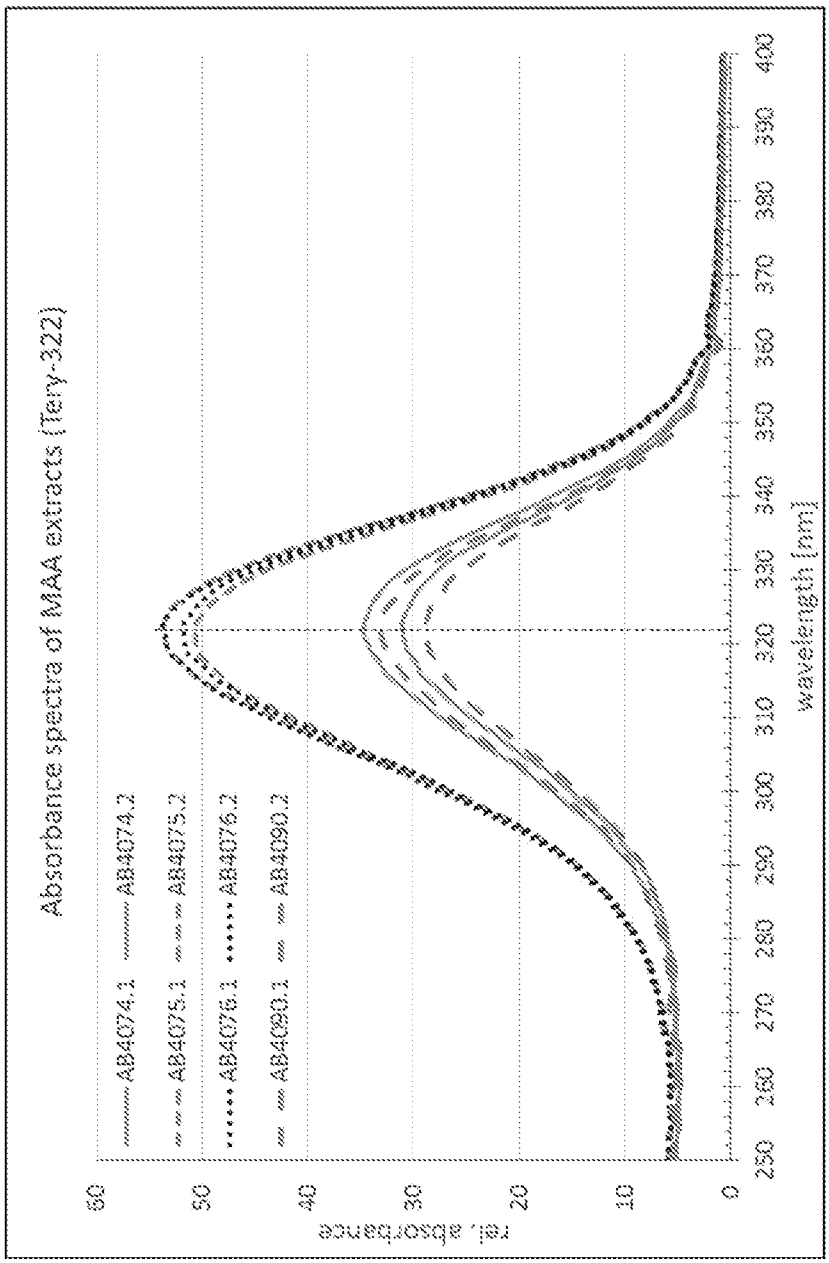

Absorbance spectra of MAA extracts (Tery-322)
AB4074.1
AB4074.2
AB4075.1
AB4075.2
AB4076.1
AB4076.2
AB4090.1
AB4090.2
rel. absorbance
wavelength [nm]
0
10
20
30
40
50
60
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400

FIG. 14

FIG. 15A

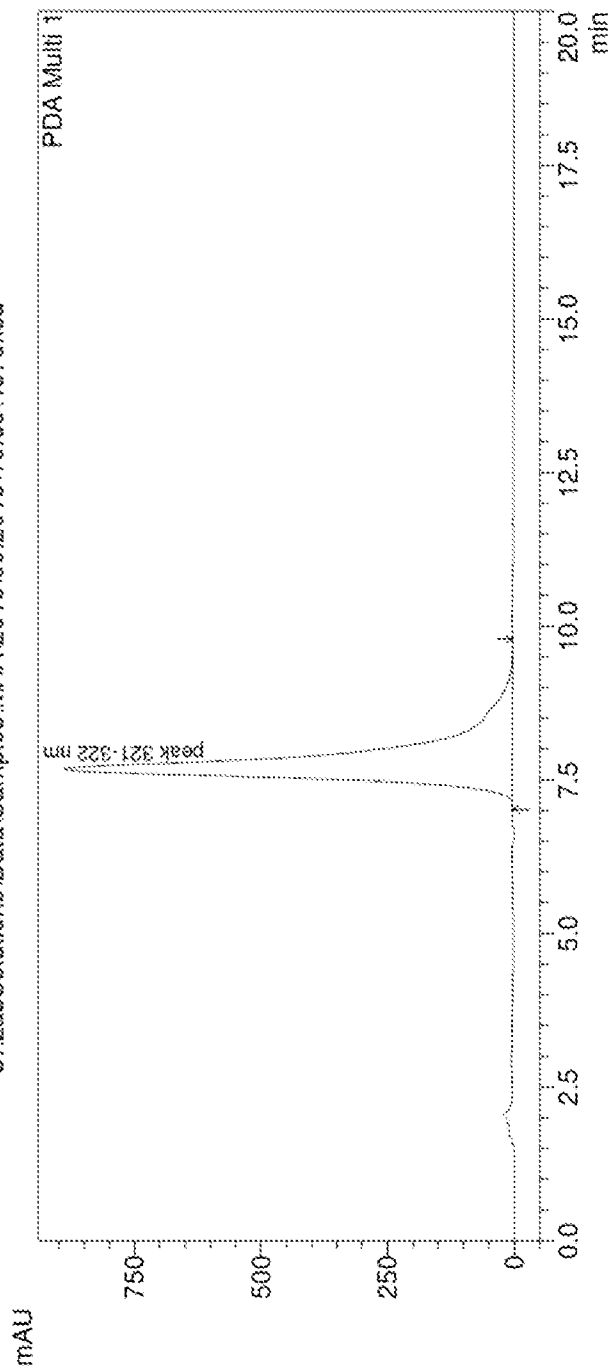
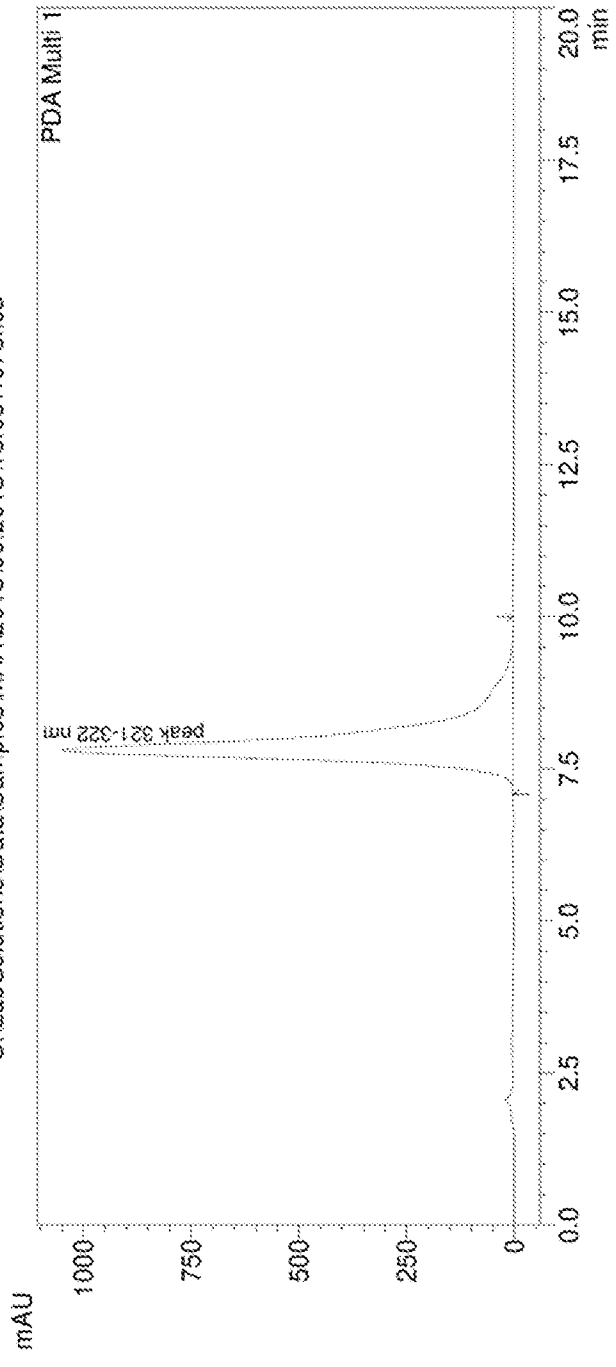
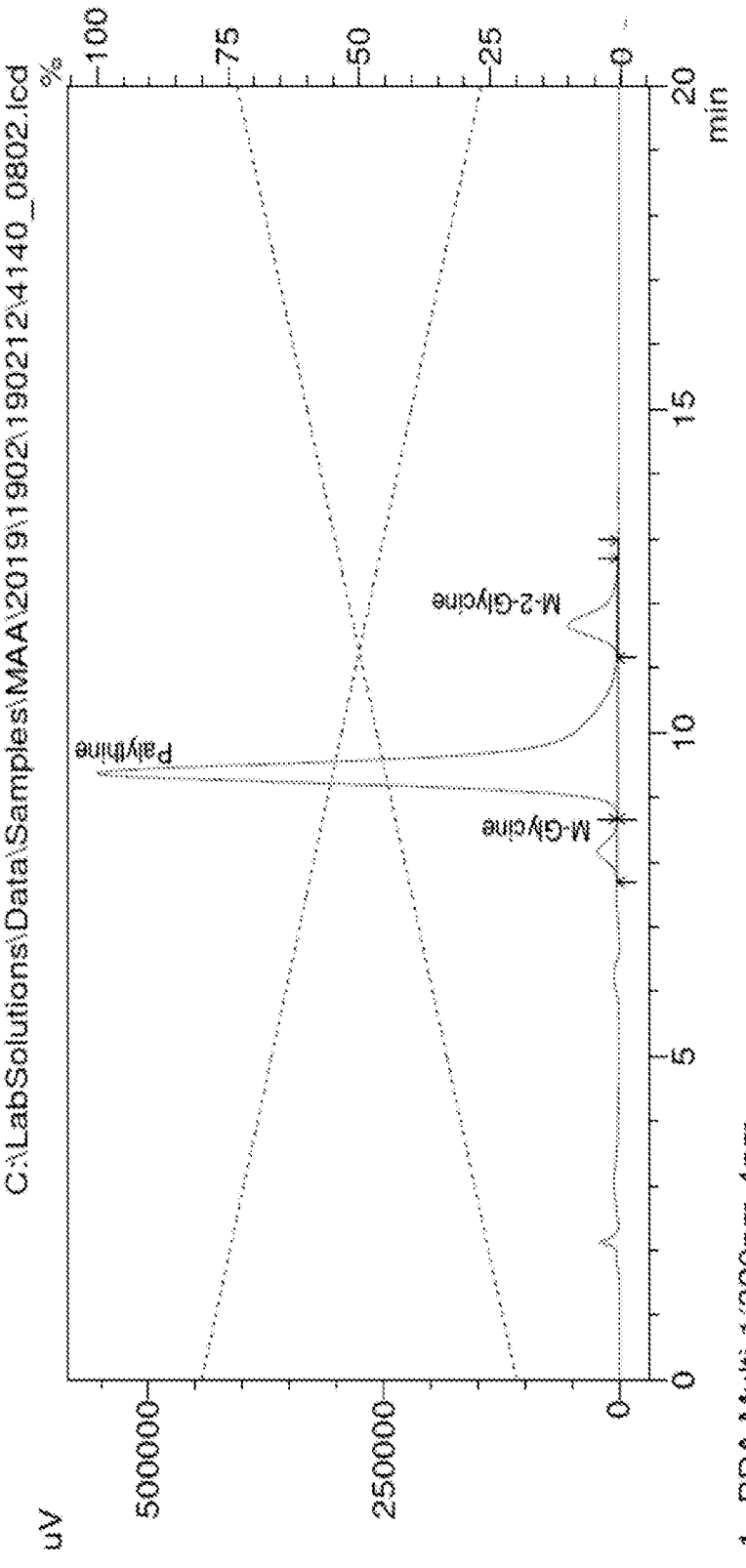


FIG. 15B





PeakTable

PDA Ch1 320nm 4nm

Name	Ret. Time	Area	Area %
M-Glycine	8.156	453984	2.6
Palythine	9.375	15388141	89.4
M-2-Glycine	11.658	1372574	8.0
		17214700	100.0

FIG. 16

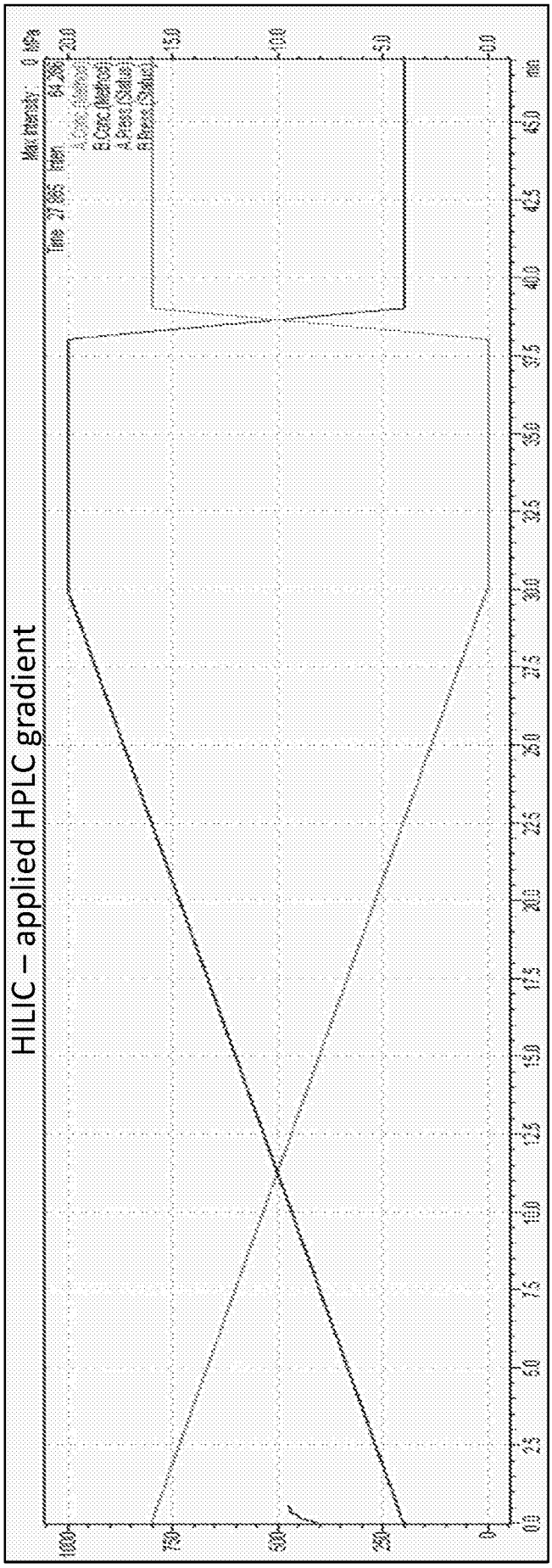

HILIC – applied HPLC gradient
Max Intensity 0 kPa
Time 27.865 min 64.286
B Conc. (Method)
A Press (Static)
B Press (Static)
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5
40.0
42.5
45.0
min
20.0
15.0
10.0
5.0
0.0

FIG. 17

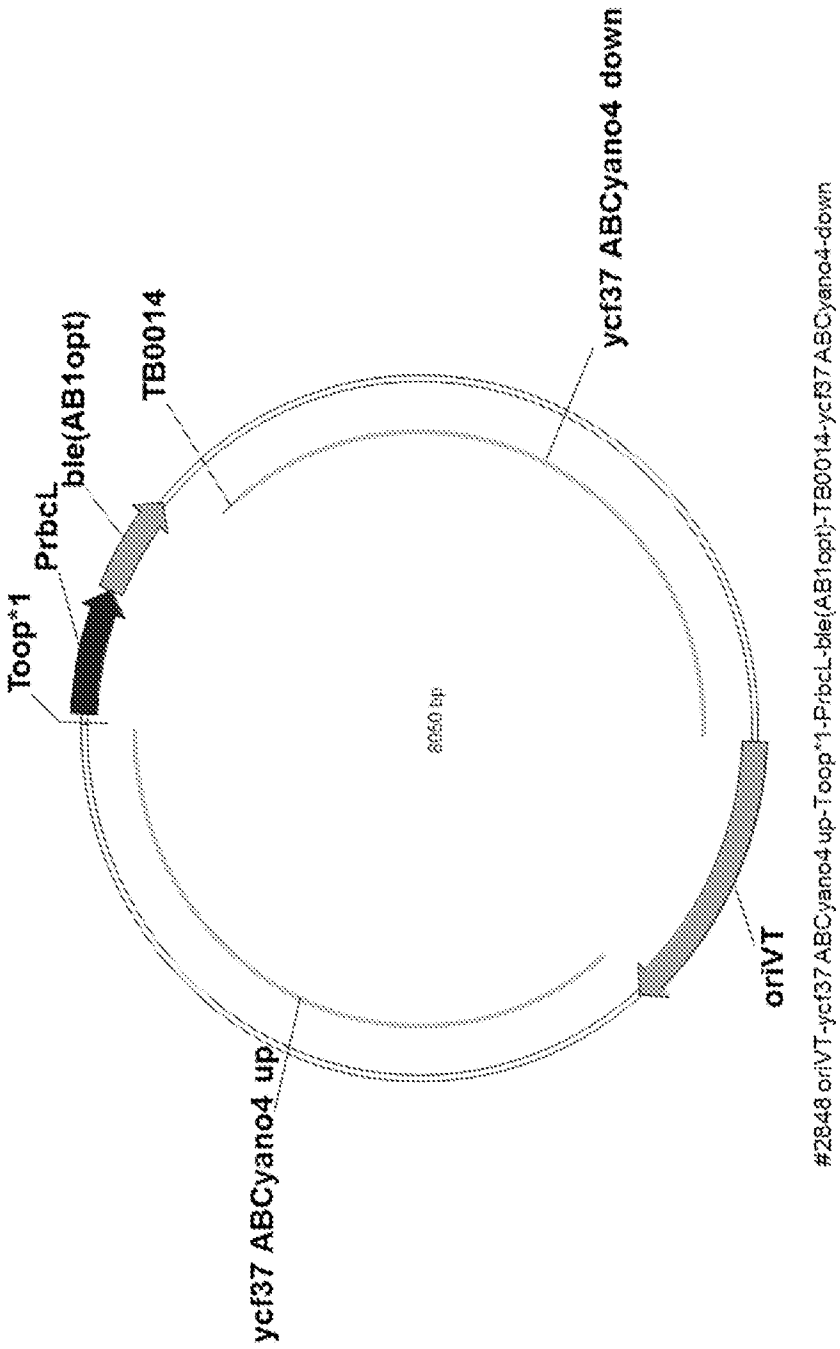

Toop*1
PrbcL
ble(AB1opt)
TB0014
ycf37 ABCyano4 down
ycf37 ABCyano4 up
oriVT
6950 bp
#2848 oriVT-ycf37 ABCyano4 up-Toop*1-PrbcL-ble(AB1opt)-TB0014-ycf37 ABCyano4-down

FIG. 18

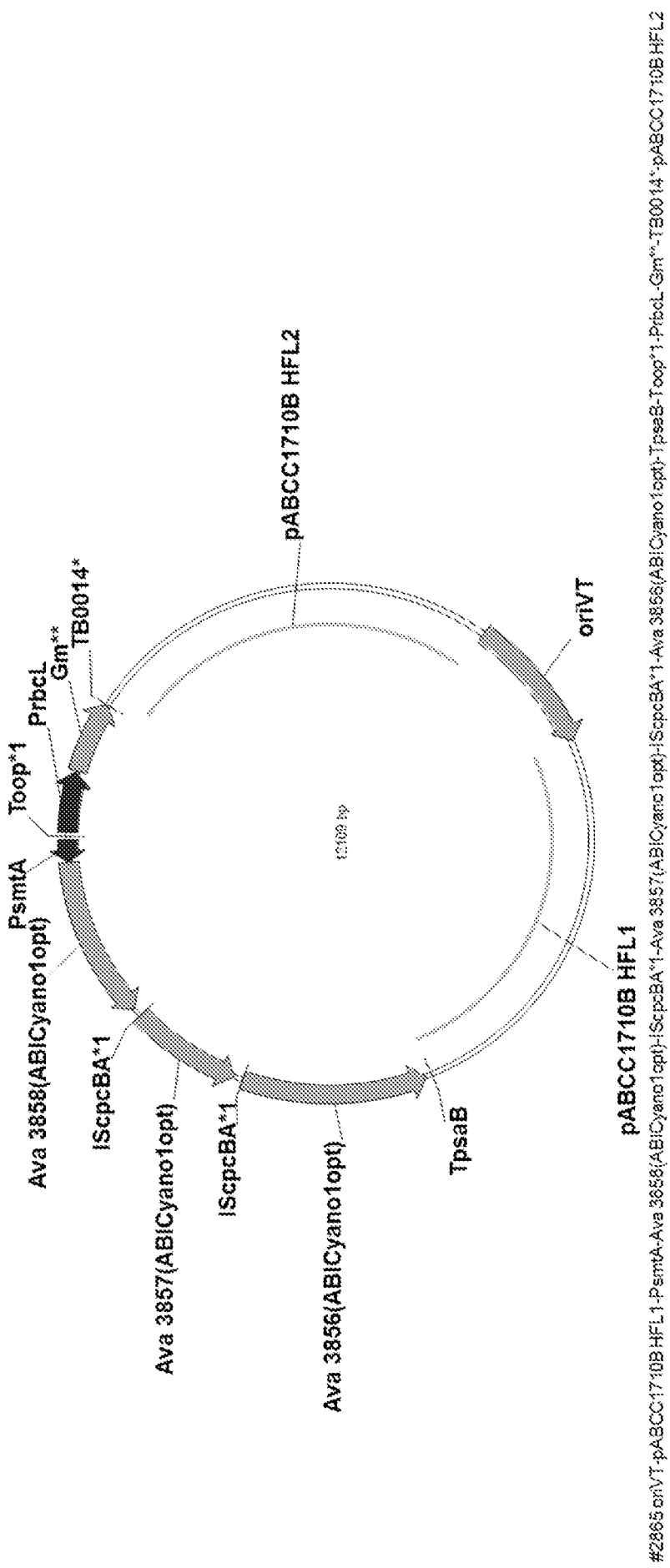

pABCC1710B HFL2
oriVT
TB0014*
Gm**
PrbcL
Toop*1
PsmtA
Ava 3858(ABICyano1opt)
IScpcBA*1
Ava 3857(ABICyano1opt)
IScpcBA*1
Ava 3856(ABICyano1opt)
TpsaB
pABCC1710B HFL1
12109 bp
#2865 oriVT-pABCC1710B HFL1-PsmtA-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-Tpsa8-Toop*1-PrbcL-Gm**-TB0014*-pABCC1710B HFL2

FIG. 19

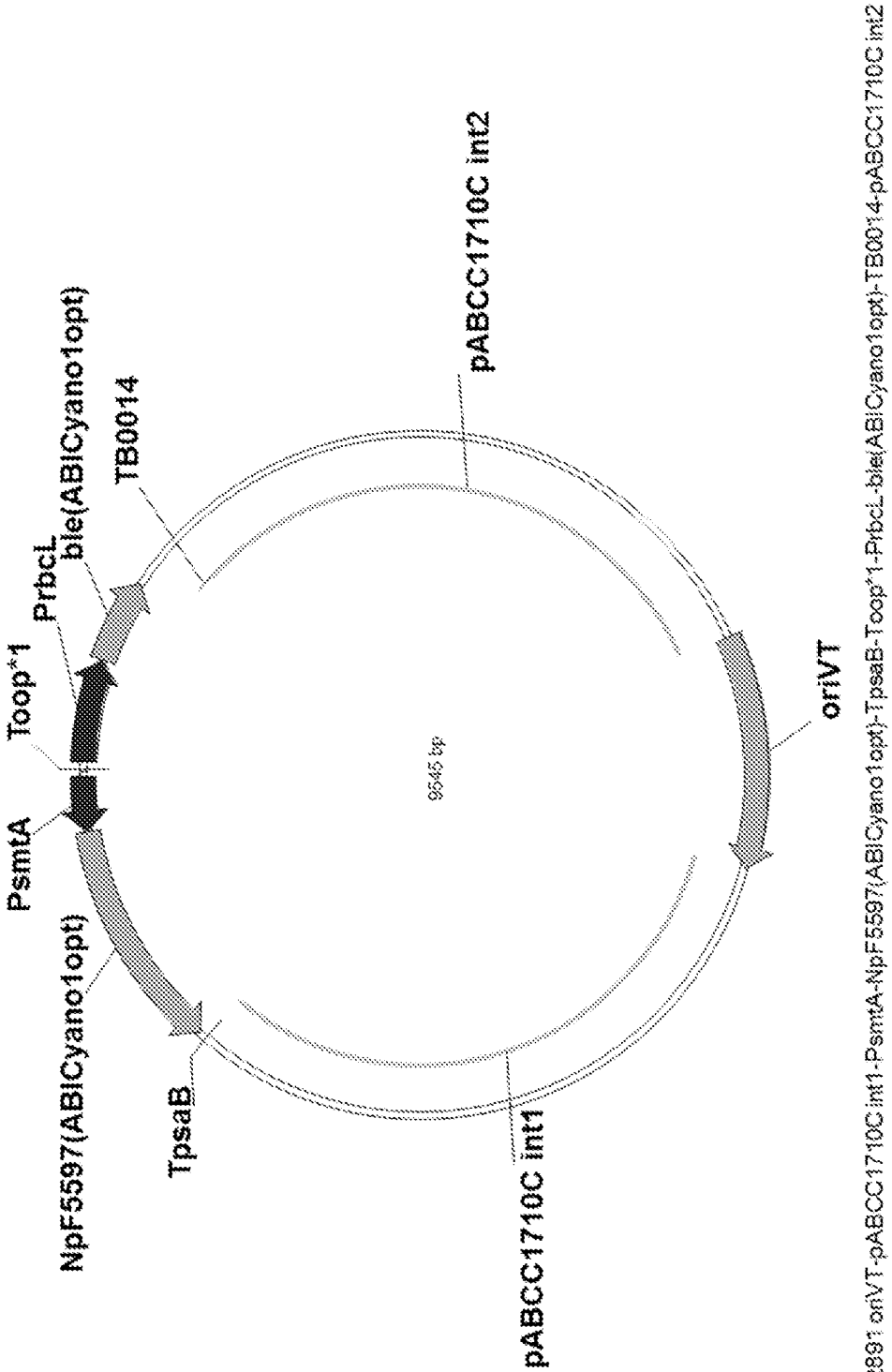

pABCC1710C int2
TB0014
ble(ABICyano1opt)
PrbcL
Toop*1
PsmtA
NpF5597(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
9545 bp
#2891 oriVT-pABCC1710C int1-PsmtA-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710C int2

FIG. 20

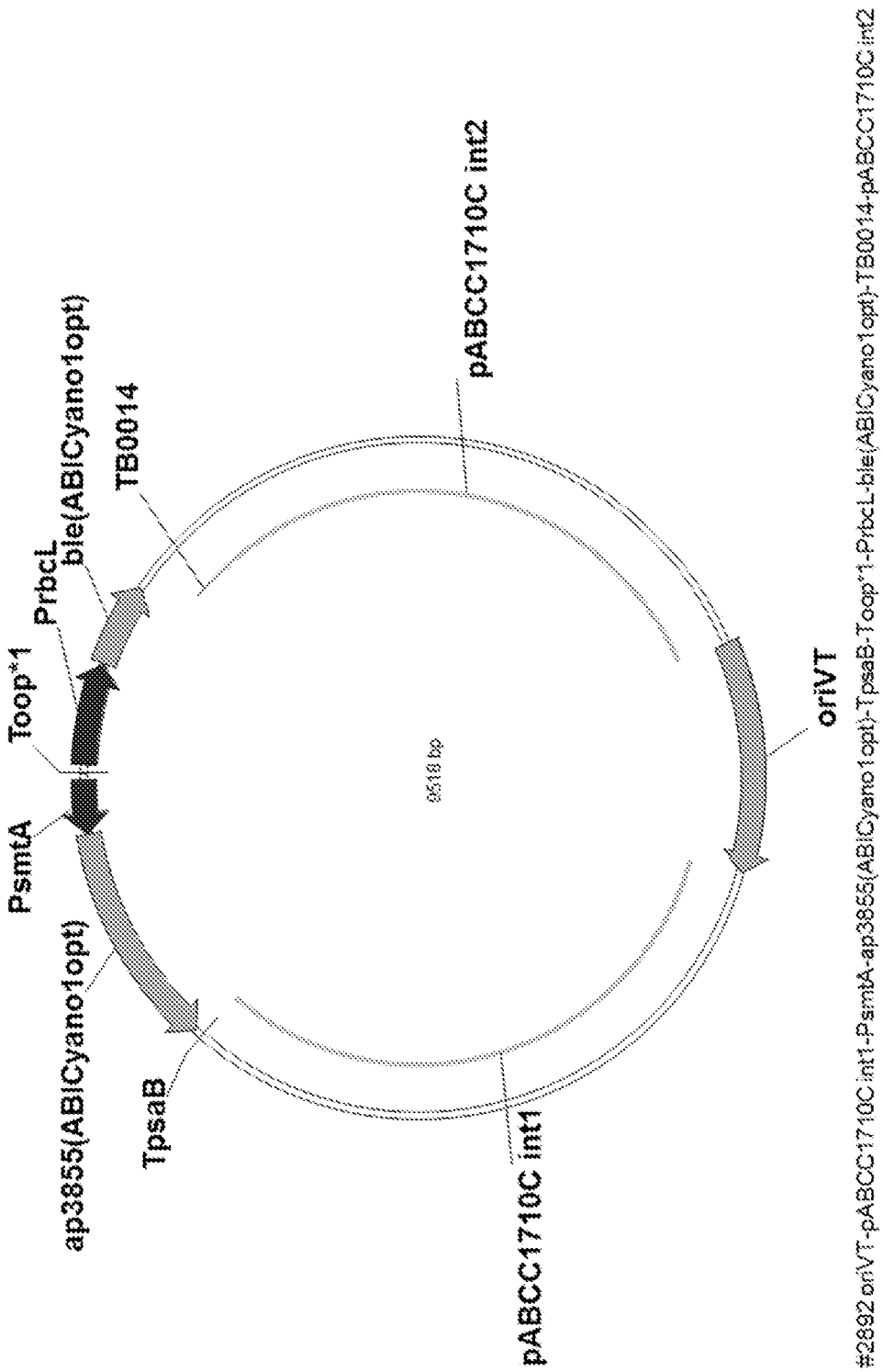

PsmtA
Toop*1
PrbcL
ble(ABICyano1opt)
TB0014
pABCC1710C int2
oriVT
ap3855(ABICyano1opt)
TpsaB
pABCC1710C int1
9518 bp
#2892 oriVT-pABCC1710C int1-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710C int2

FIG. 21

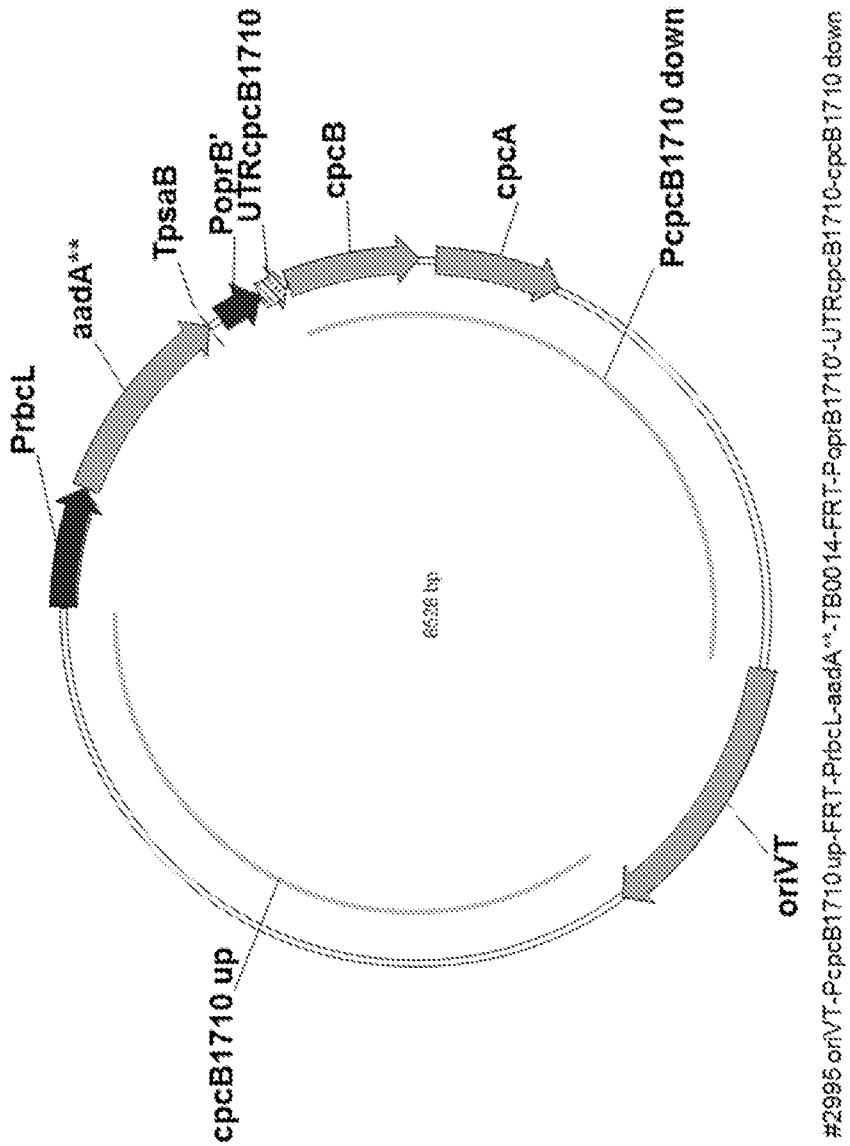

PrbcL
aadA**
TpsaB
PoprB'
UTRcpcB1710
cpcB
cpcA
PcpcB1710 down
oriVT
cpcB1710 up
#2996 oriVT-PcpcB1710 up-FRT-PrbcL-aadA**-TB0014-FRT-PoprB1710'-UTRcpcB1710-cpcB1710 down

FIG. 22

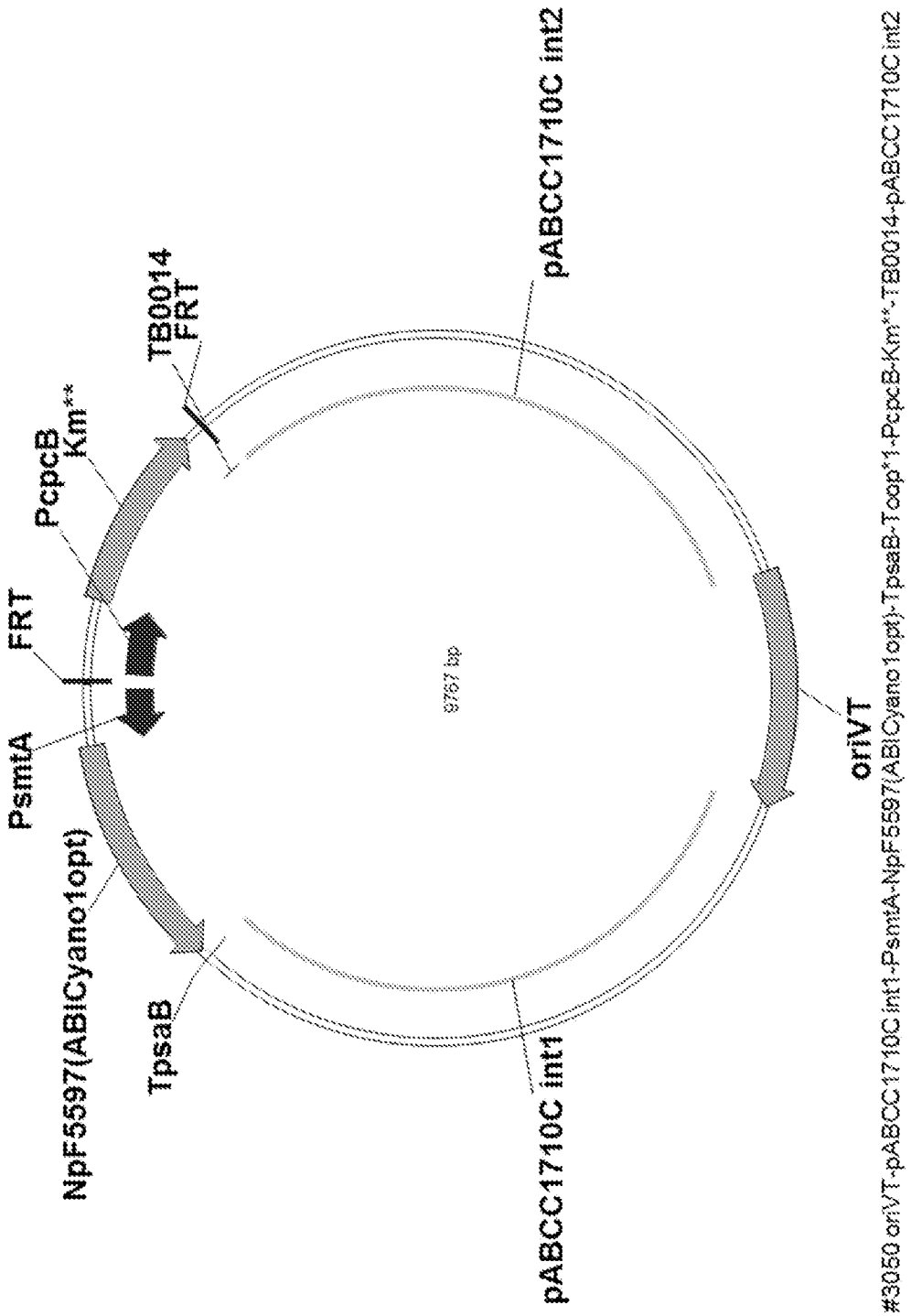

9767 bp
pABCC1710C int2
TB0014 FRT
PcpcB
Km**
FRT
PsmtA
NpF5597(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
#3050 oriVT-pABCC1710C int1-PsmtA-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PcpcB-Km**-TB0014-pABCC1710C int2

FIG. 23

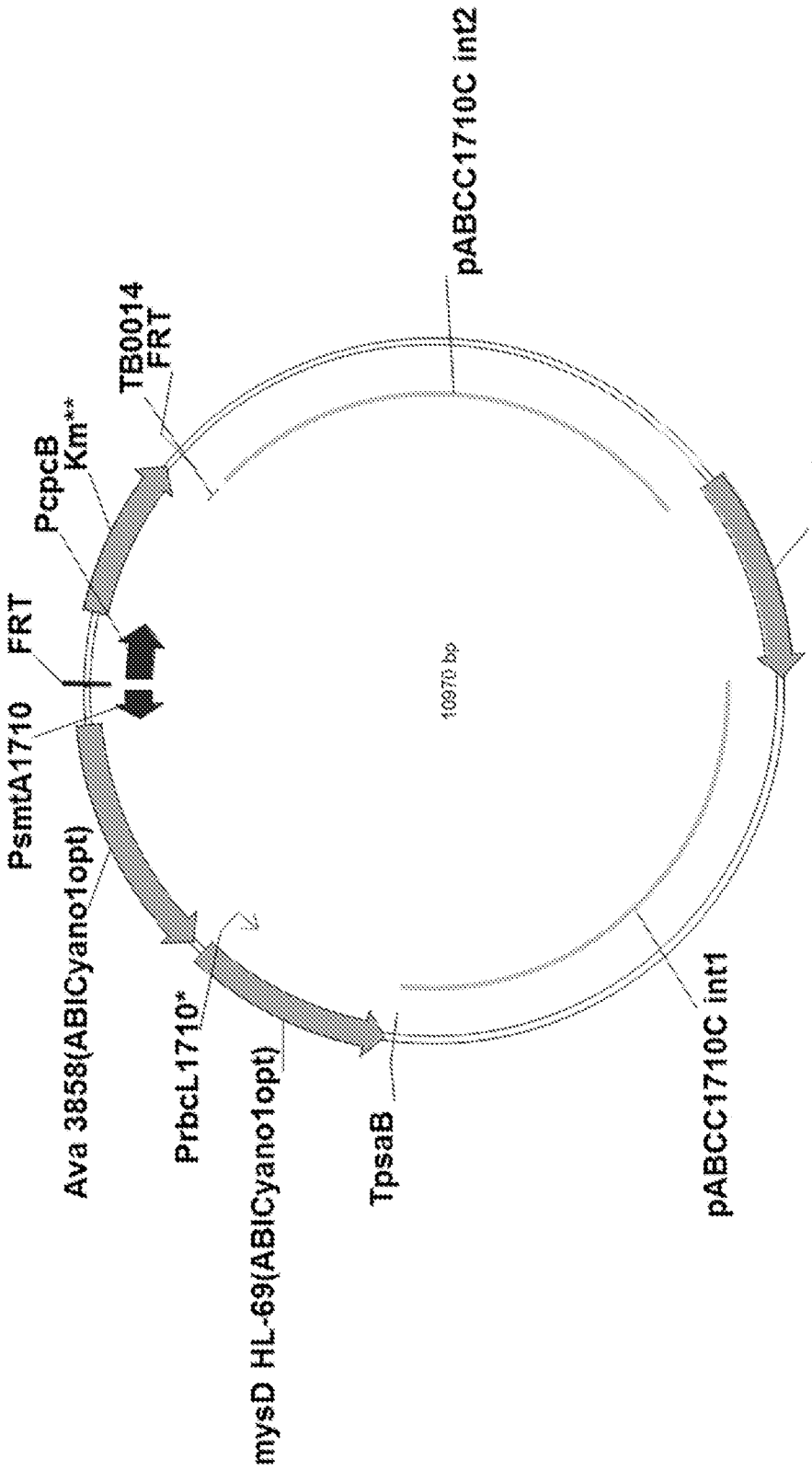

pABCC1710C int2
TB0014 FRT
PcpcB Km**
FRT
PsmtA1710
Ava 3858(ABICyano1opt)
PrbcL1710*
mysD HL-69(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
10970 bp
#3075 oriVT-pABCC1710C int1 HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-PrbcL1710*-mysD HL-69(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 24

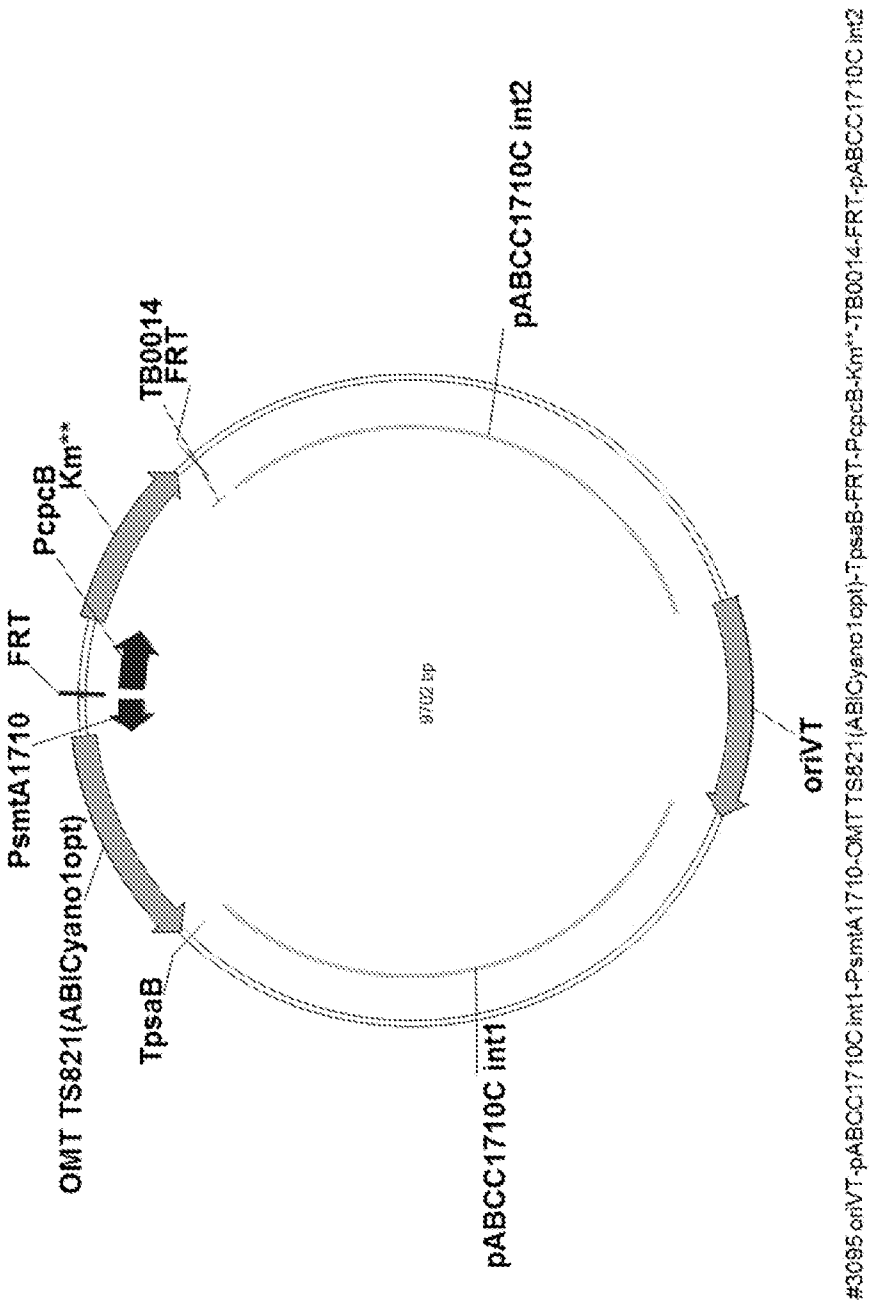

pABCC1710C int2
TB0014 FRT
PcpcB
Km**
FRT
PsmtA1710
OMT TS821(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
9702 bp
#3095 oriVT-pABCC1710C int1-PsmtA1710-OMT TS821(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 25

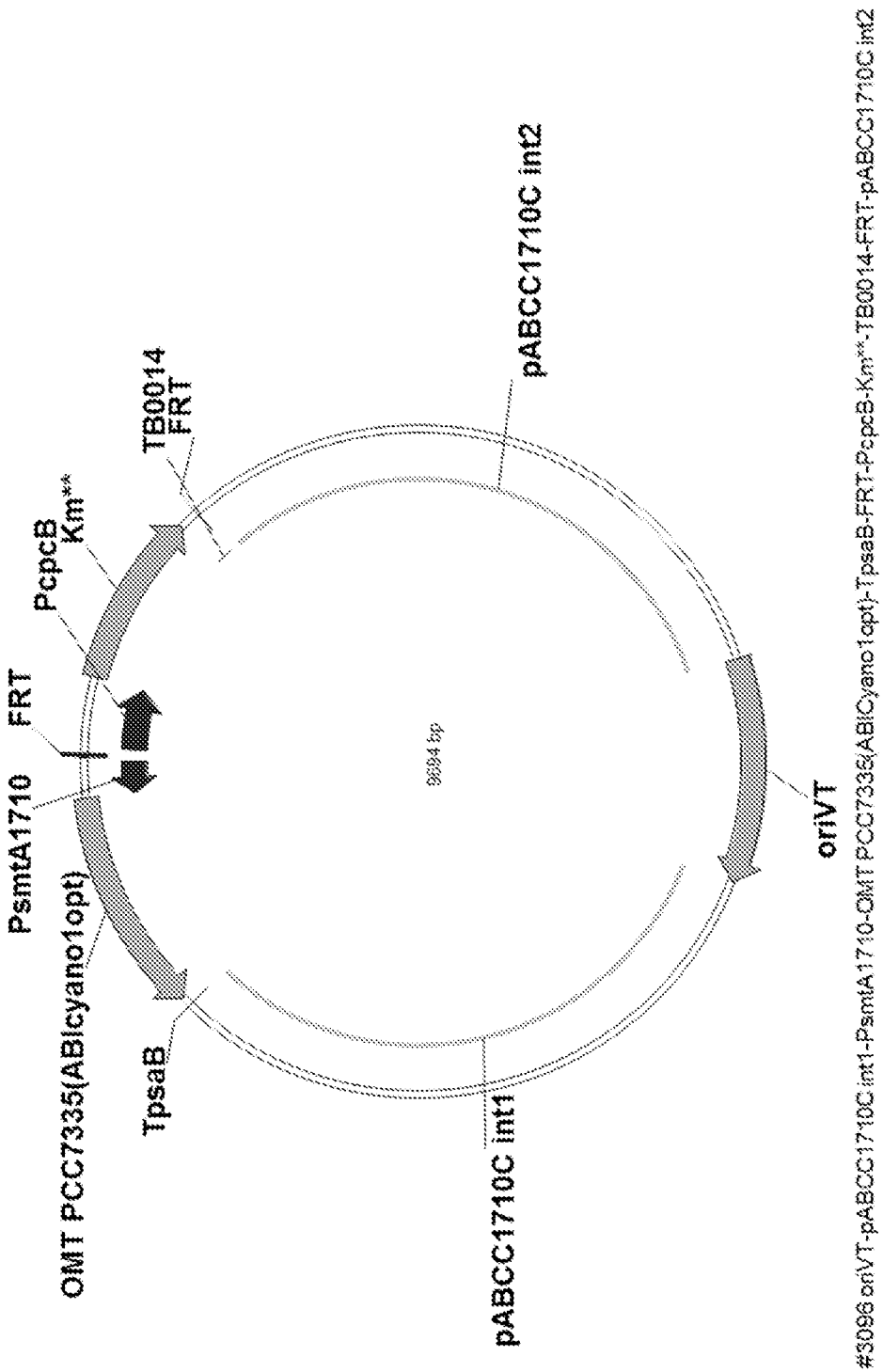

pABCC1710C int2
TB0014 FRT
PcpcB Km**
FRT
PsmtA1710
OMT PCC7335(ABIcyano1opt)
TpsaB
pABCC1710C int1
oriVT
9894 bp
#3096 oriVT-pABCC1710C int1-PsmtA1710-OMT PCC7335(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 26

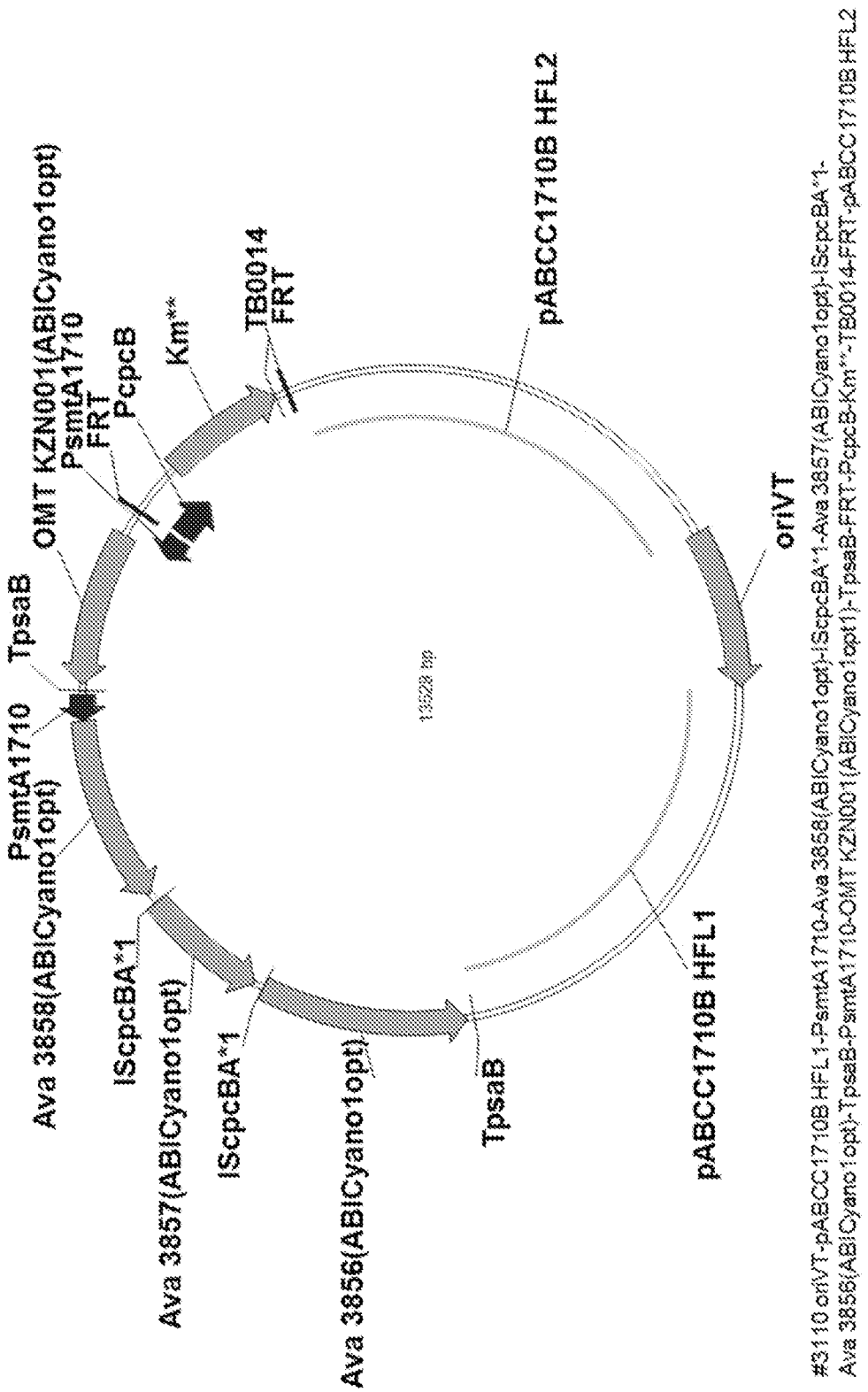

PsmtA1710
Ava 3858(ABICyano1opt)
TpsaB
OMT KZN001(ABICyano1opt)
PsmtA1710
FRT
PcpcB
Km**
TB0014
FRT
pABCC1710B HFL2
oriVT
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
Ava 3856(ABICyano1opt)
TpsaB
pABCC1710B HFL1
13528 bp
#3110 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-lScpcBA*1-Ava 3857(ABICyano1opt)-lScpcBA*1-
Ava 3856(ABICyano1opt)-TpsaB-PsmtA1710-OMT KZN001(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710B HFL2

FIG. 27

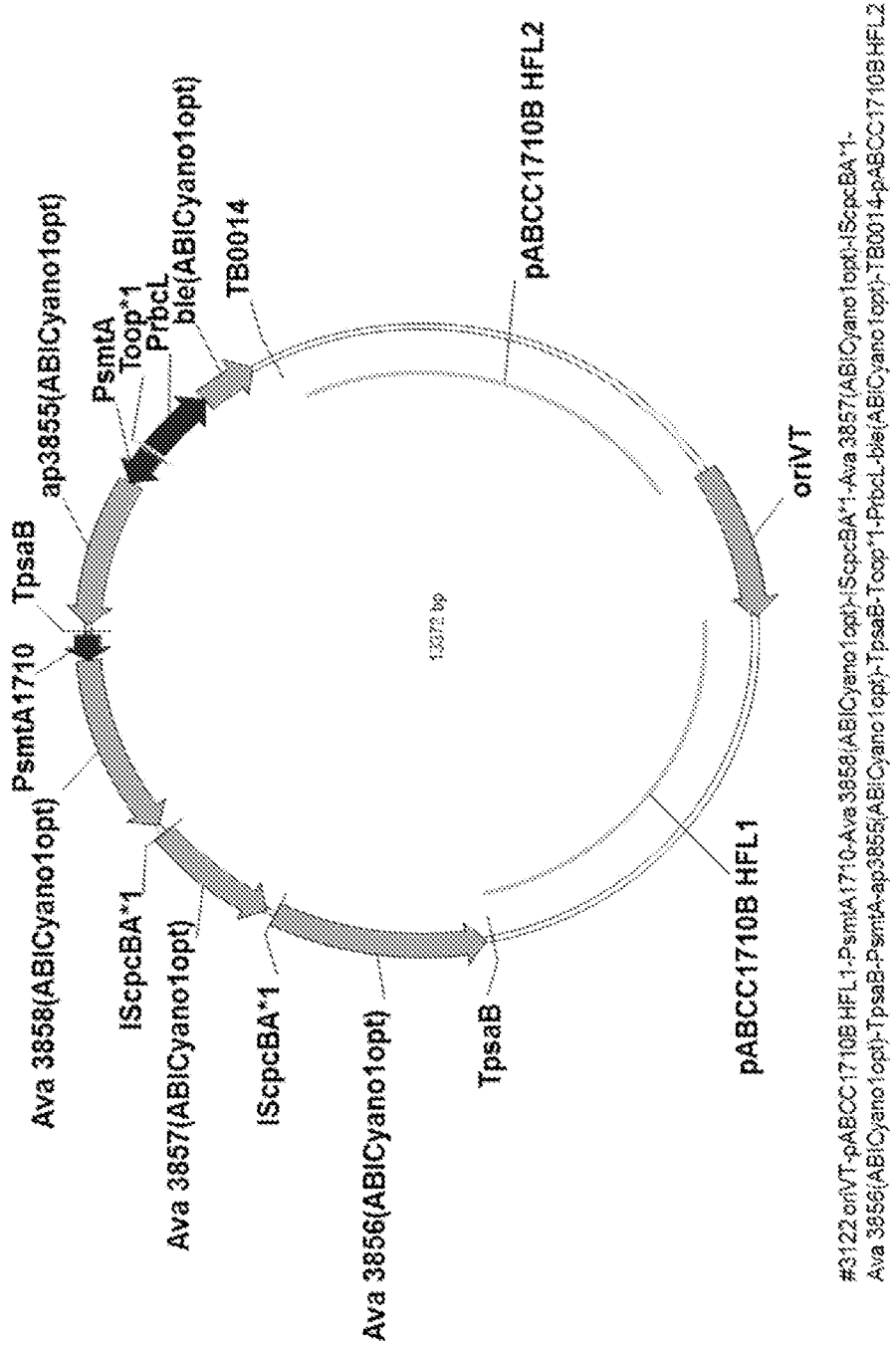

ap3855(ABICyano1opt)
PsmtA
Toop*1
PrbcL
ble(ABICyano1opt)
TB0014
pABCC1710B HFL2
oriVT
TpsaB
PsmtA1710
Ava 3858(ABICyano1opt)
IScpcBA*1
Ava 3857(ABICyano1opt)
IScpcBA*1
Ava 3856(ABICyano1opt)
TpsaB
pABCC1710B HFL1
#3122 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-
Ava 3856(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B HFL2

FIG. 28

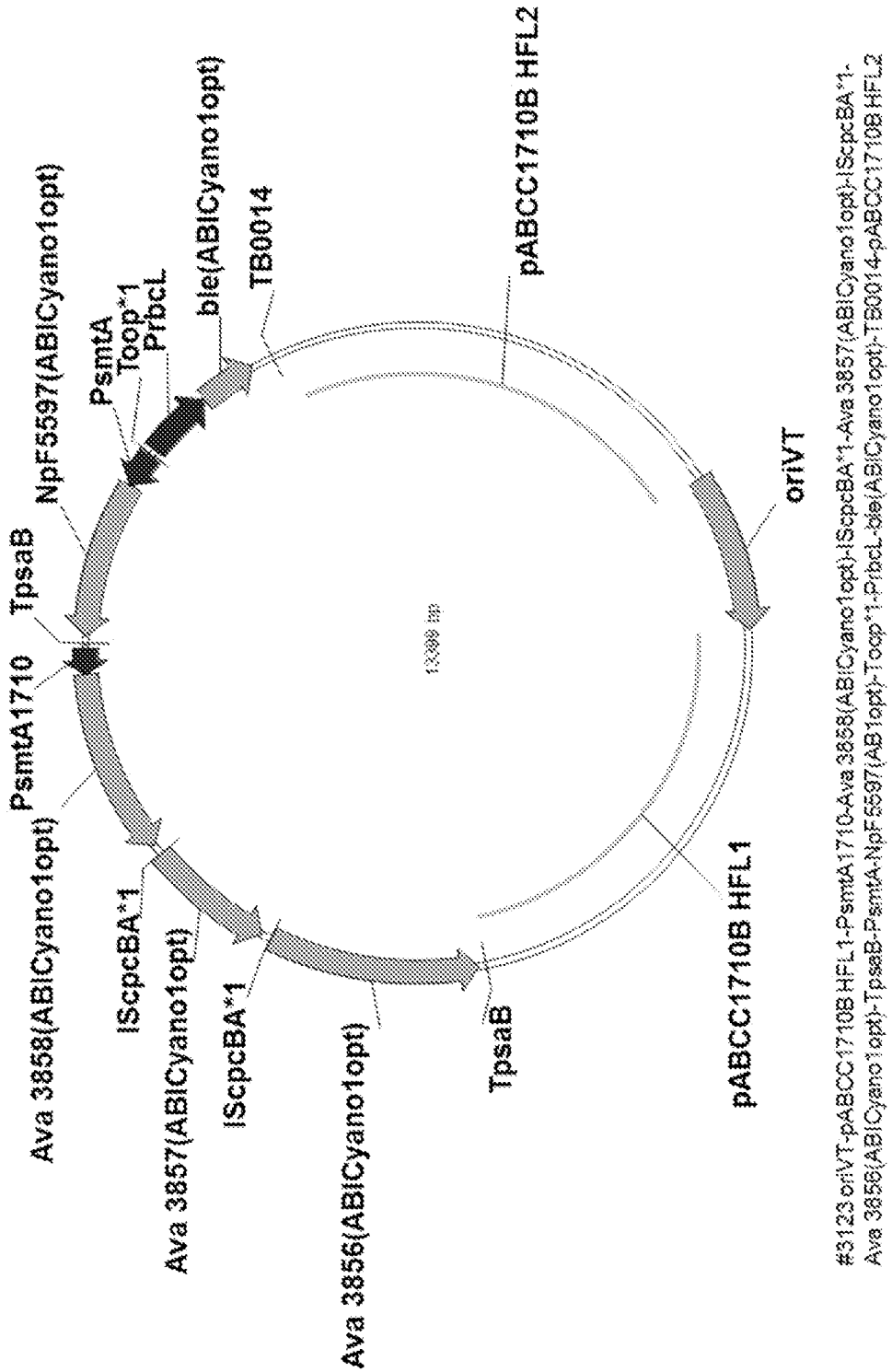

Ava 3858(ABICyano1opt)
PsmtA1710
TpsaB
NpF5597(ABICyano1opt)
PsmtA
Toop*1
PrbcL
ble(ABICyano1opt)
TB0014
pABCC1710B HFL2
oriVT
pABCC1710B HFL1
TpsaB
Ava 3856(ABICyano1opt)
IScpcBA*1
Ava 3857(ABICyano1opt)
IScpcBA*1
#3123 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-TpsaB-PsmtA-NpF5597(AB1opt)-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B HFL2

FIG. 29

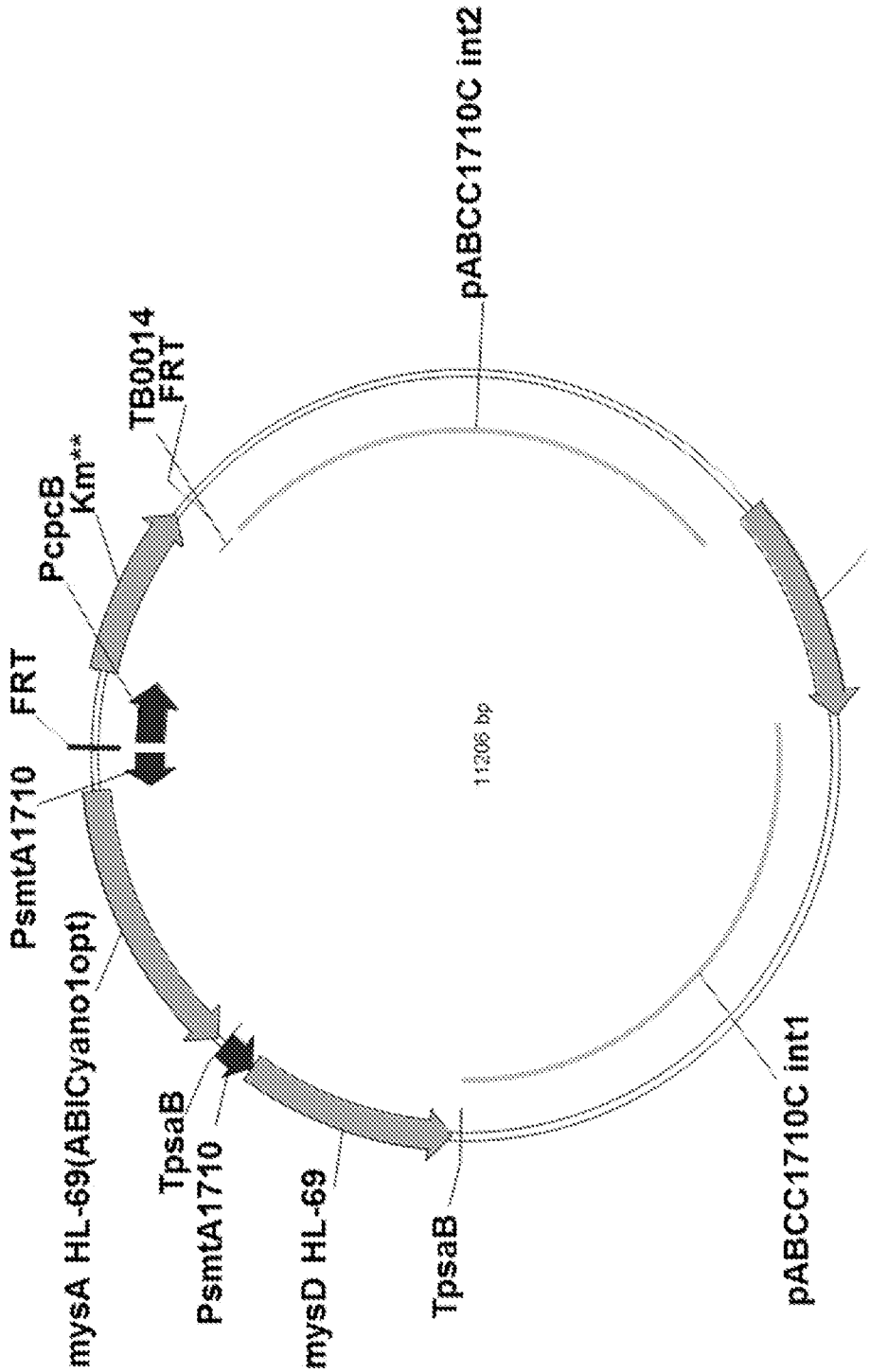

pABCC1710C int2
TB0014 FRT
PcpcB Km**
FRT
PsmtA1710
mysA HL-69(ABICyano1opt)
TpsaB
PsmtA1710
mysD HL-69
TpsaB
pABCC1710C int1
oriVT
11306 bp
#3125 oriVT-pABCC1710C int1-PsmtA1710-mysA HL-69(AB1opt)-PsmtA1710-mysD HL-69(AB1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 30

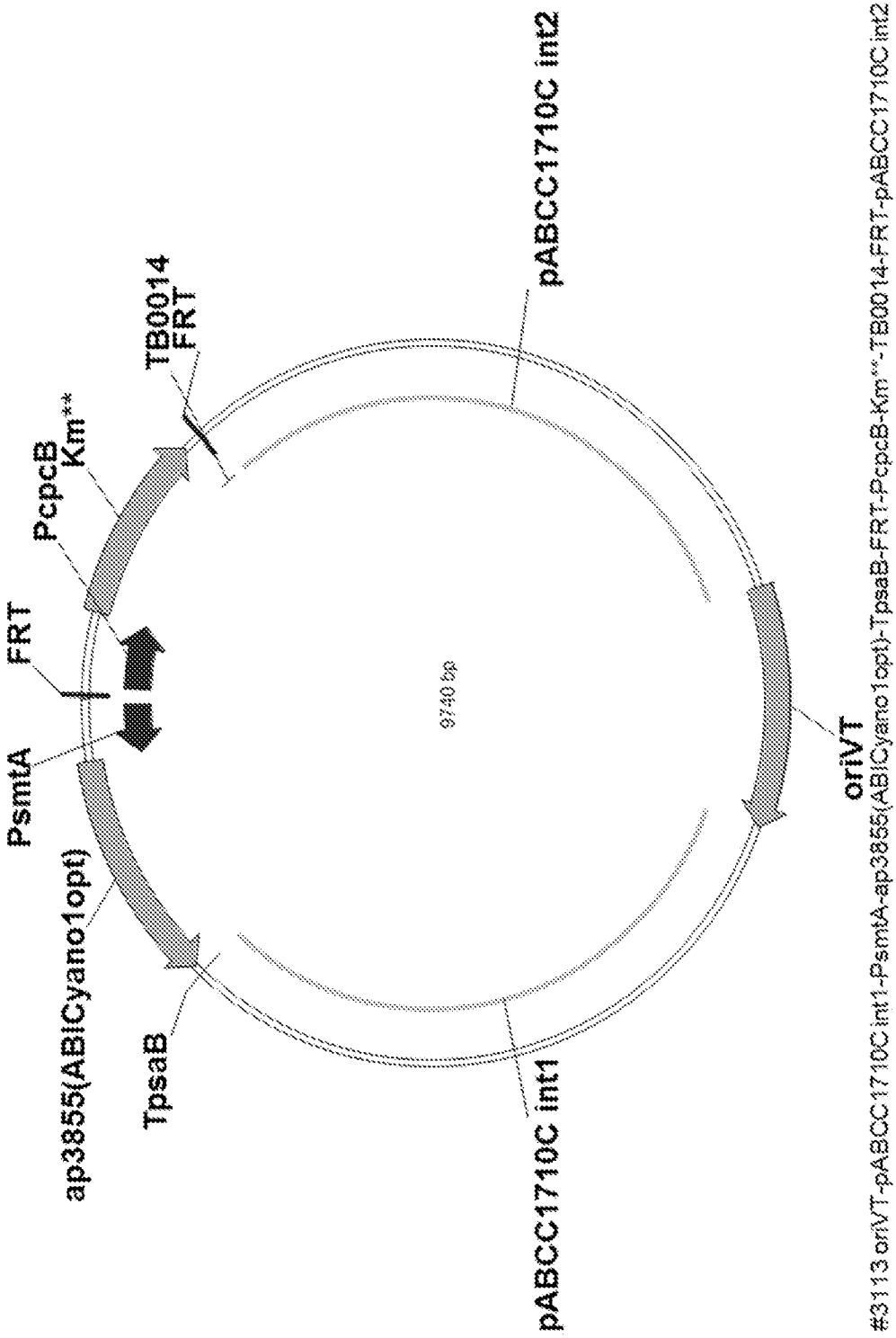

pABCC1710C int2
TB0014
FRT
PcpcB
Km**
FRT
PsmtA
ap3855(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
9740 bp
#3113 oriVT-pABCC1710C int1-PsmtA-ap3855(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 31

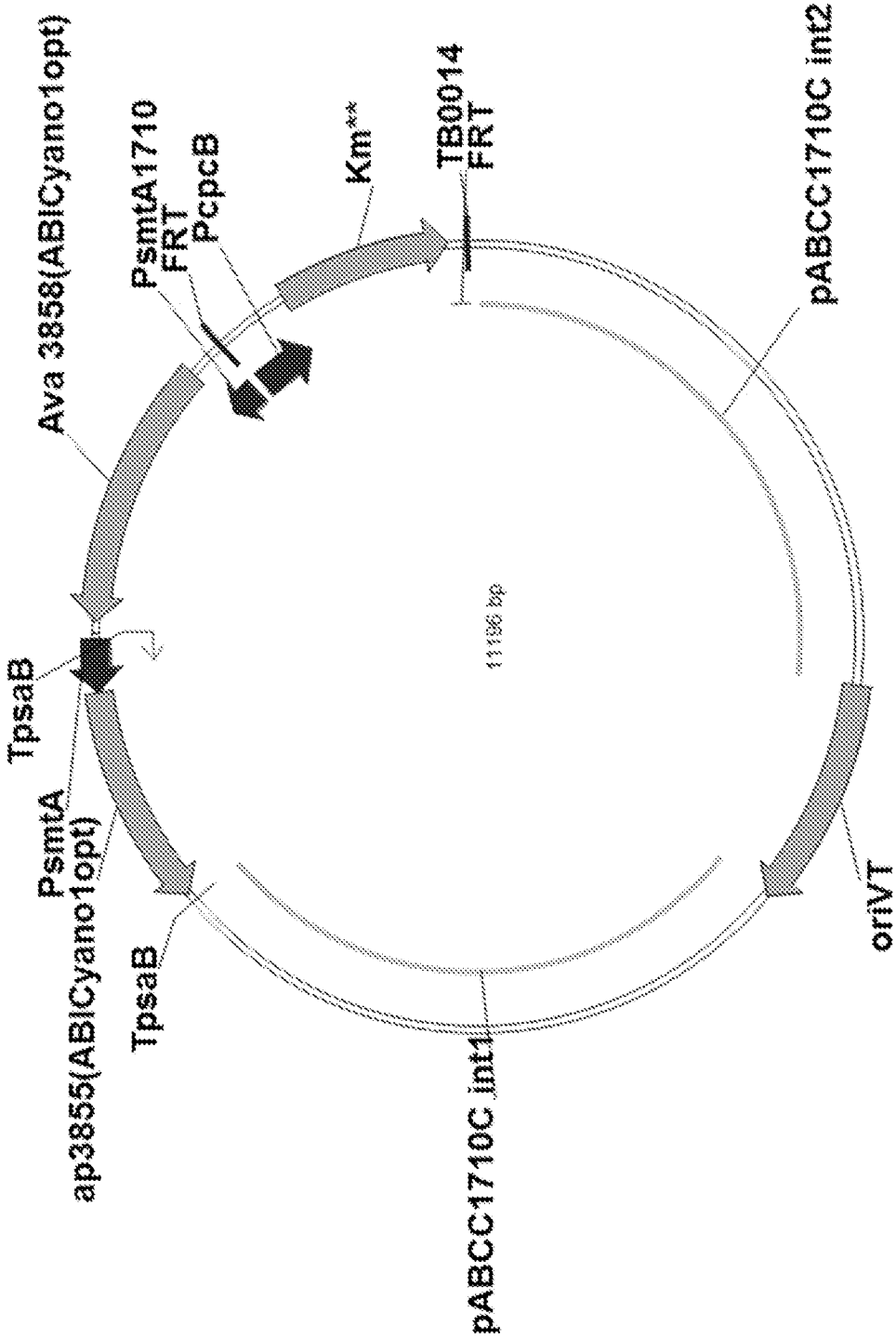

Ava 3858(ABICyano1opt)
PsmtA1710
FRT
PcpcB
Km**
TB0014
FRT
pABCC1710C int2
TpsaB
PsmtA
ap3855(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
11196 bp
#3140 oriVT-pABCC1710C int1-PsmtA1710-Ava 3858(ABICyano1opt)-TpsaB-PsmtA-ap3855(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 32

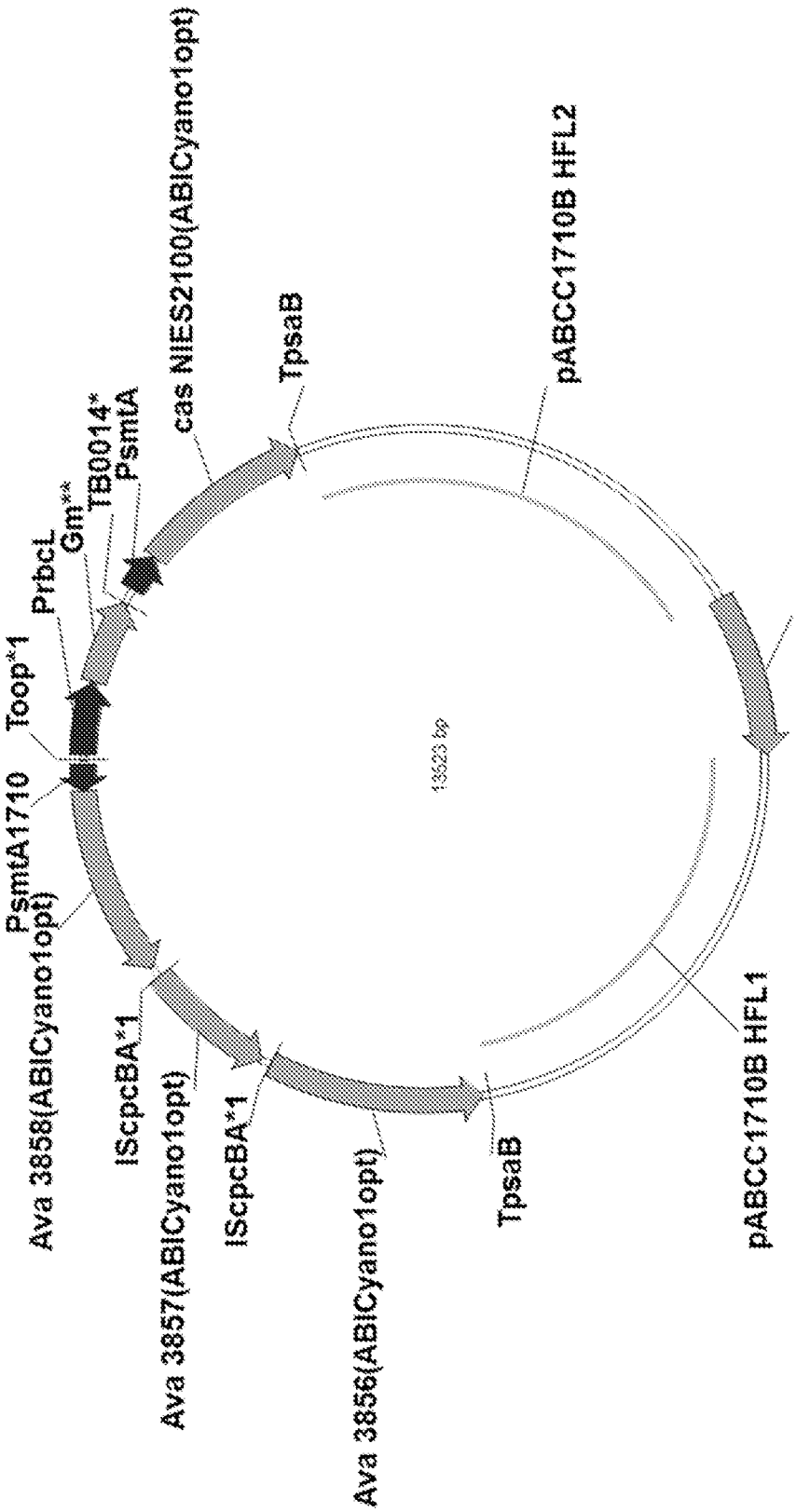

PsmtA1710
Toop*1
PrbcL
Gm**
TB0014*
PsmtA
cas NIES2100(ABICyano1opt)
TpsaB
pABCC1710B HFL2
oriVT
pABCC1710B HFL1
TpsaB
Ava 3856(ABICyano1opt)
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
Ava 3858(ABICyano1opt)
13523 bp
#3190 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(AB1opt)-lScpcBA*1-Ava 3857(AB1opt)-lScpcBA*1-Ava 3856(AB1opt)-TpsaB-PsmtA-cas NIES2100(AB1opt)-TpsaB-PrbcL-Gm**-TB0014*-pABCC1710B HFL2

FIG. 33

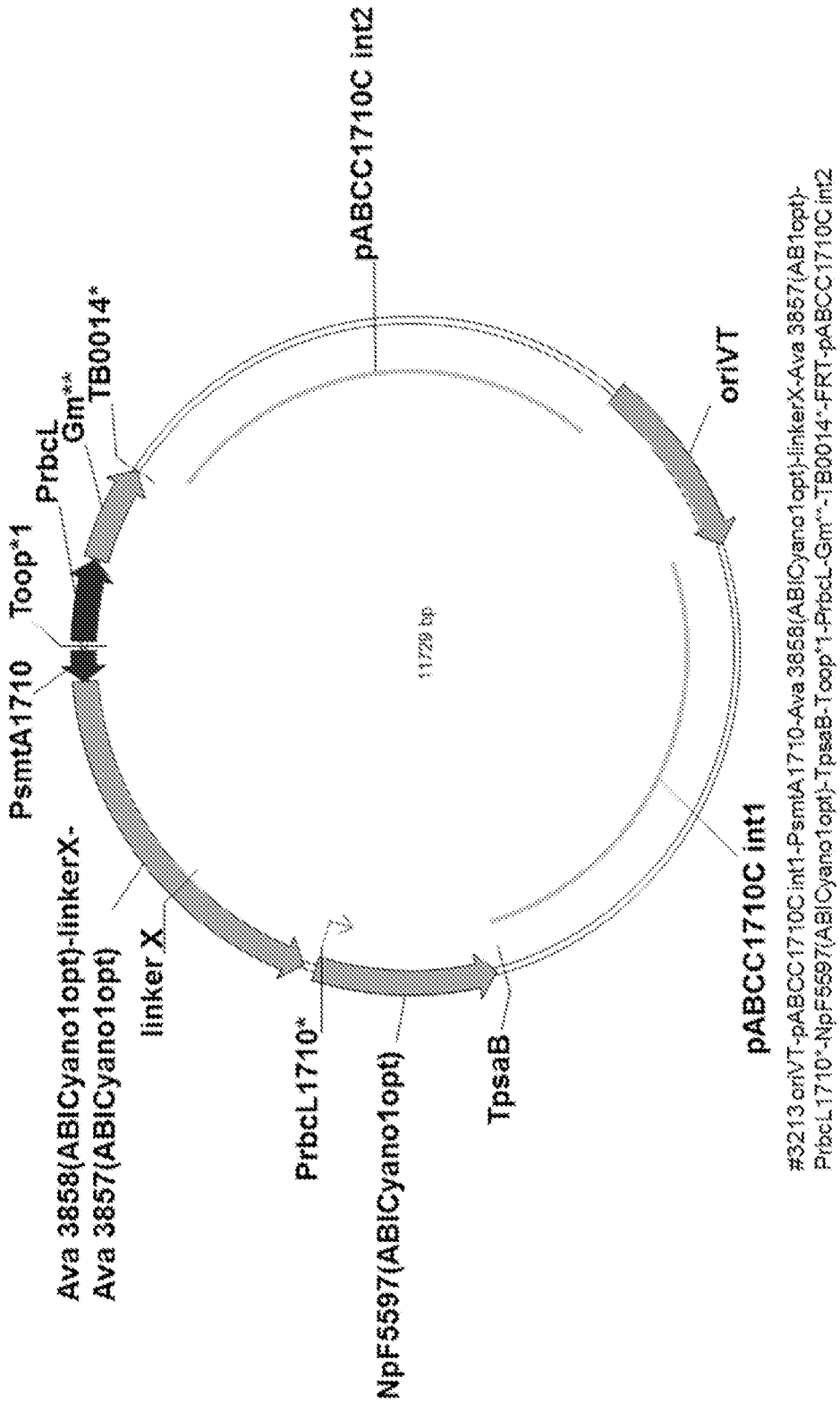
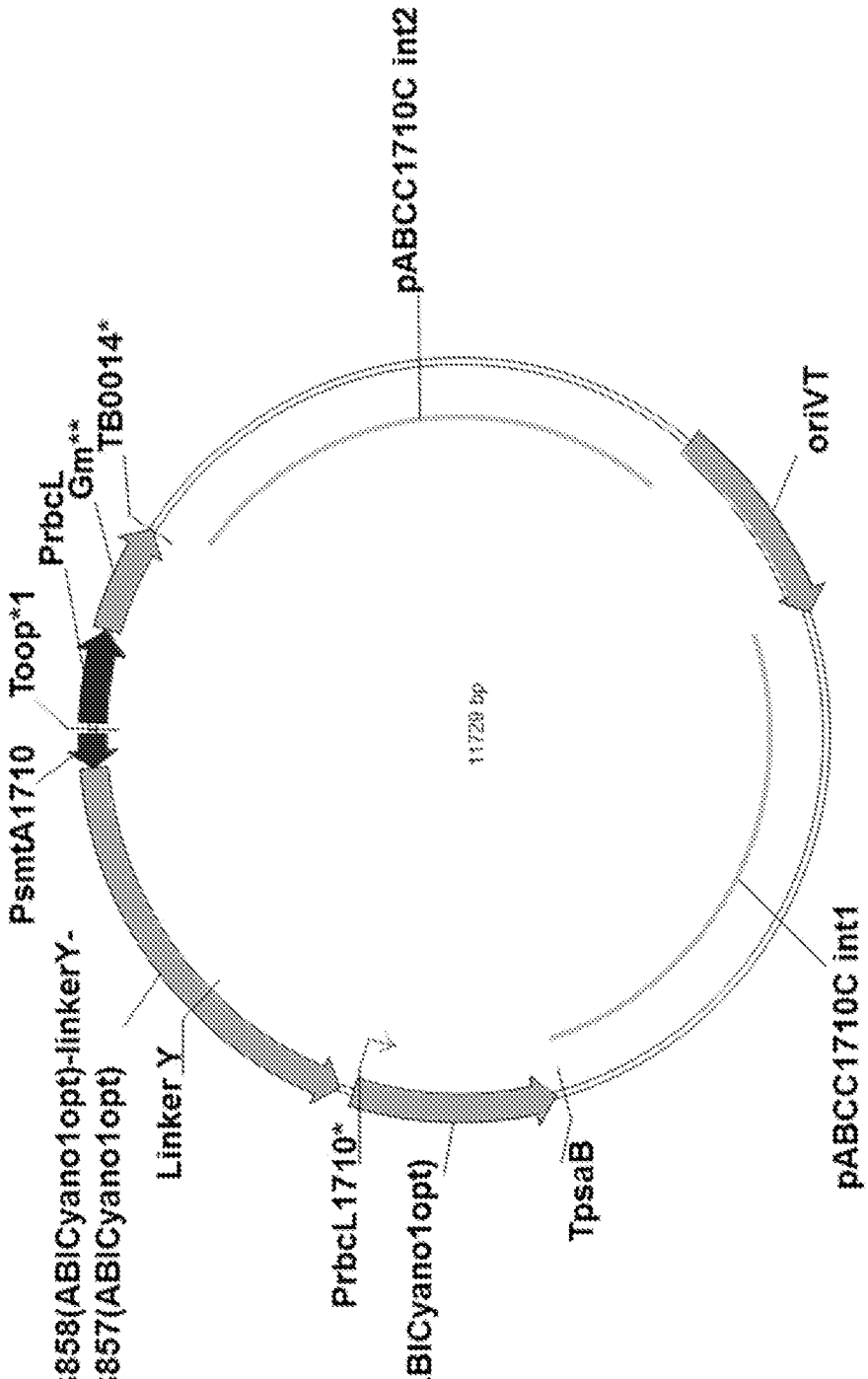

pABCC1710C int2
TB0014*
Gm**
PrbcL
Toop*1
PsmtA1710
Ava 3858(ABICyano1opt)-linkerX-
Ava 3857(ABICyano1opt)
linker X
PrbcL1710*
NpF5597(ABICyano1opt)
TpsaB
pABCC1710C int1
orIVT
11729 bp
#3213 orIVT-pABCC1710C int1-PsmtA1710-Ava 3858(ABICyano1opt)-linkerX-Ava 3857(AB1opt)-PrbcL1710*-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-FRT-pABCC1710C int2

FIG. 34



#32140riVT-pABCC1710C int1-PsmtA1710-Ava 3858(ABICyano1opt)-linkerY-Ava 3857(ABICyano1opt)-PrbcL1710*-NpF5597(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-FRT-pABCC1710C int2

FIG. 35

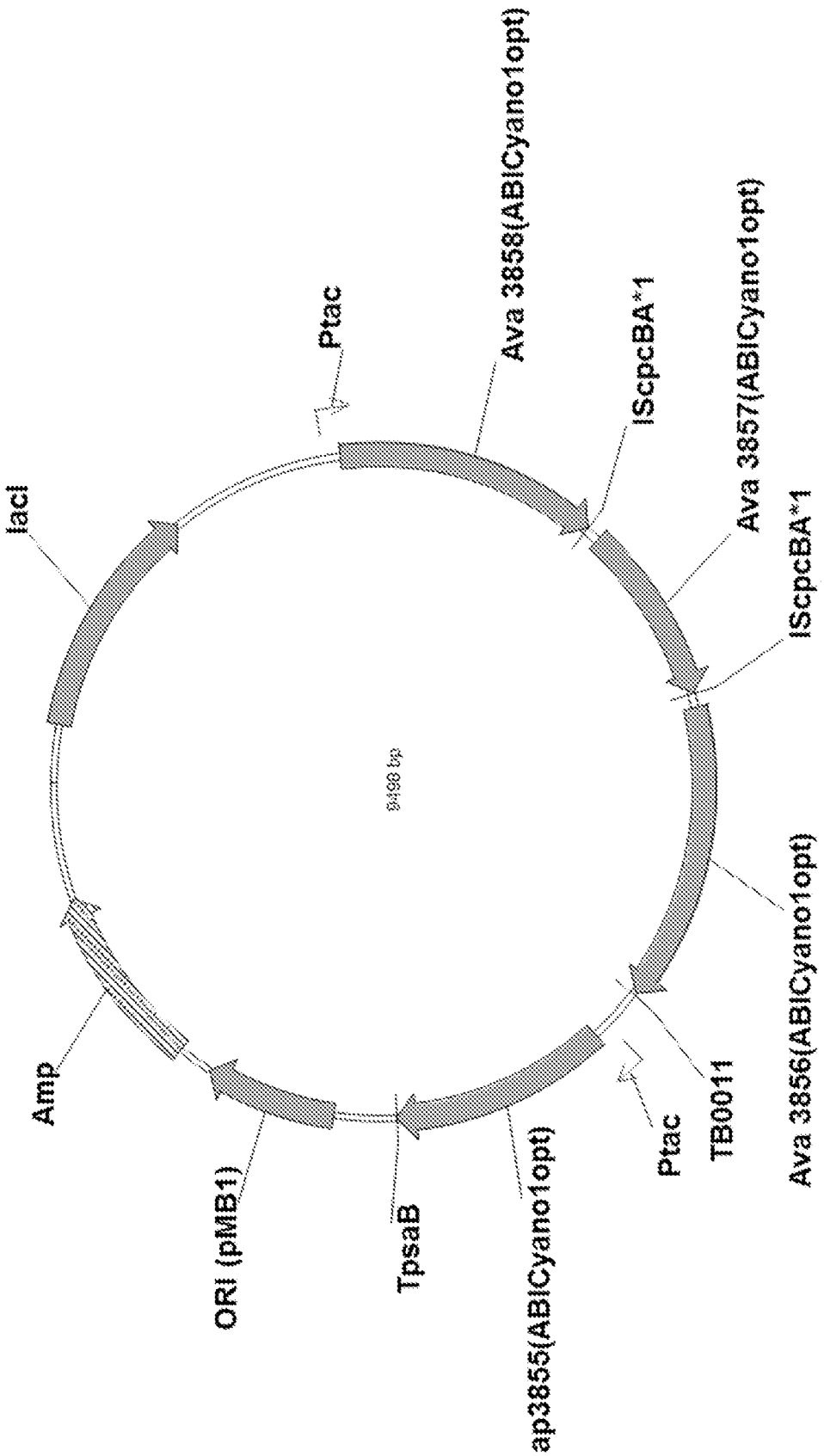

lacI
Ptac
Ava 3858(ABICyano1opt)
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
Ava 3856(ABICyano1opt)
TB0011
Ptac
ap3855(ABICyano1opt)
TpsaB
ORI (pMB1)
Amp
8498 bp
#3130 pJet1.2::lacI-Ptac-Ava 3858(ABICyano1opt)-lScpcBA*1-Ava 3857(ABICyano1opt)-lScpcBA*1-Ava 3856(ABICyano1opt)-TB0011-Ptac-ap3855(ABICyano1opt)-TpsaB

FIG. 36

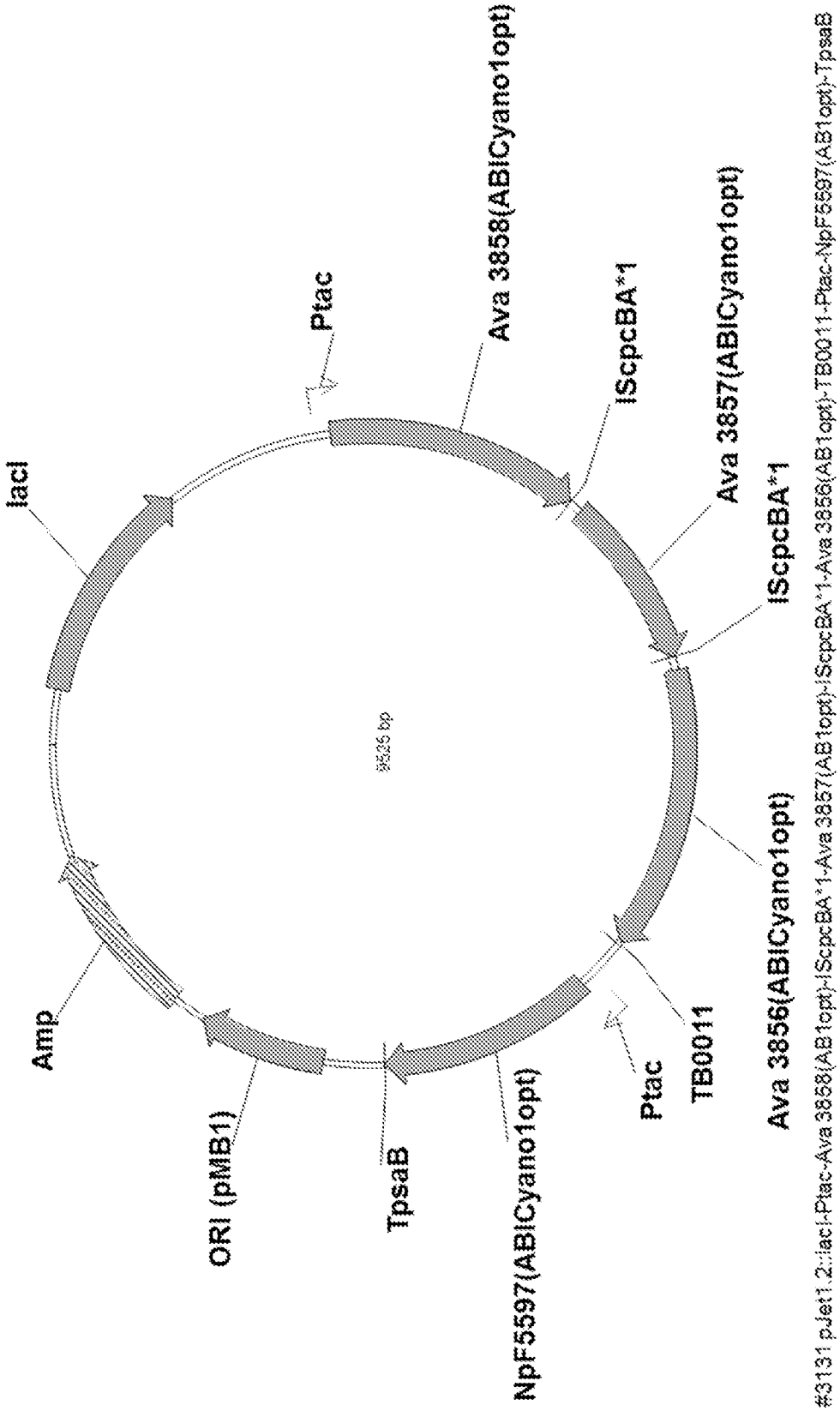

lacI
Ptac
Ava 3858(ABICyano1opt)
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
Ava 3856(ABICyano1opt)
TB0011
Ptac
NpF5597(ABICyano1opt)
TpsaB
ORI (pMB1)
Amp
9535 bp
#3131 pJet1.2::lacI-Ptac-Ava 3858(AB1opt)-lScpcBA*1-Ava 3857(AB1opt)-lScpcBA*1-Ava 3856(AB1opt)-TB0011-Ptac-NpF5597(AB1opt)-TpsaB

FIG. 37

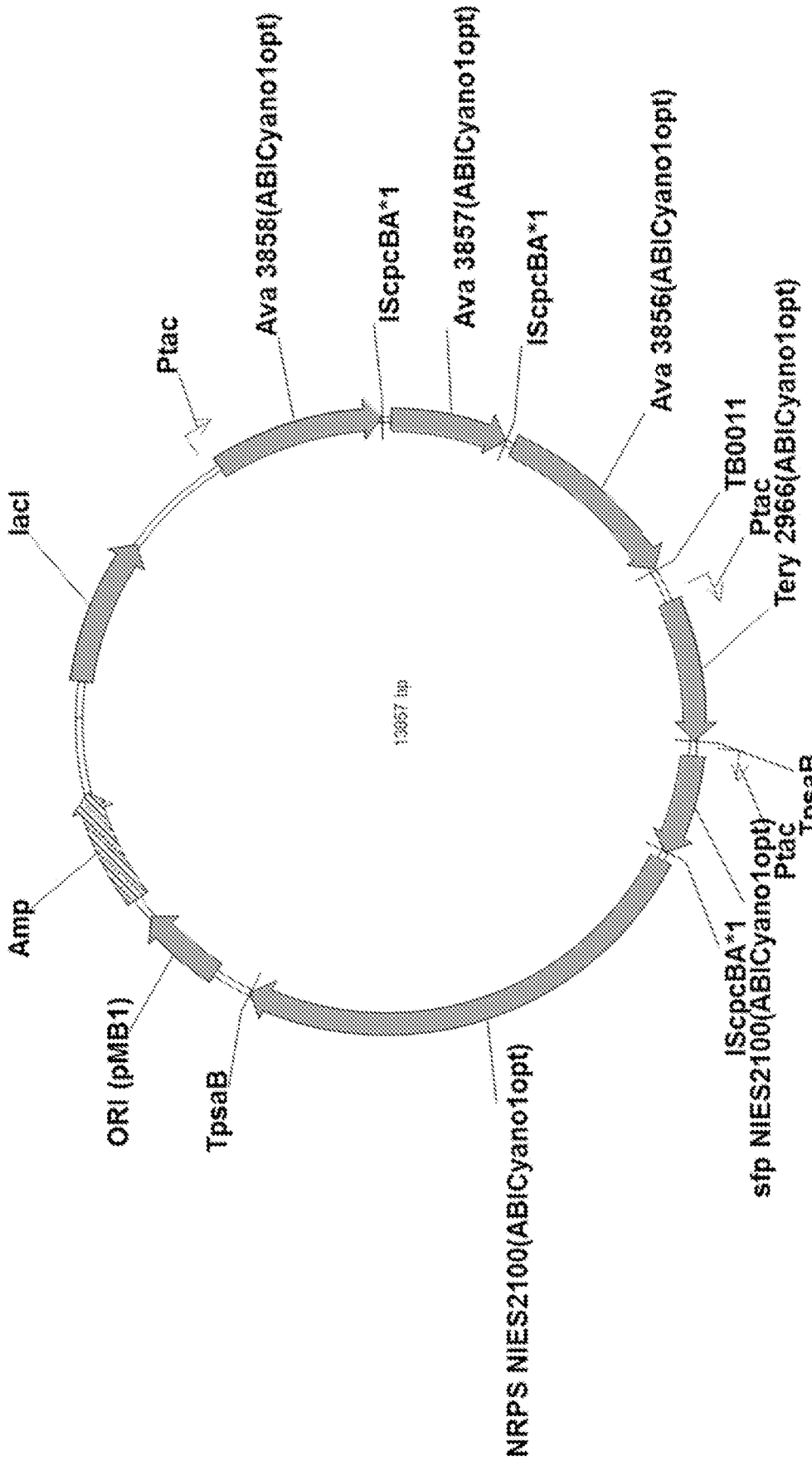

lacI
Ptac
Ava 3858(ABICyano1opt)
IScpcBA*1
Ava 3857(ABICyano1opt)
IScpcBA*1
Ava 3856(ABICyano1opt)
TB0011
Ptac
Tery 2966(ABICyano1opt)
TpsaB
Ptac
IScpcBA*1
sfp NIES2100(ABICyano1opt)
NRPS NIES2100(ABICyano1opt)
TpsaB
ORI (pMB1)
Amp
13857 bp
#3186 pJet1.2::lacI-Ptac-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-TB0011-Ptac-Tery 2966(ABICyano1opt)-TpsaB-Ptac-sfp NIES2100(ABICyano1opt)-IScpcBA*1-NRPS NIES2100(ABICyano1opt)-TpsaB

FIG. 38

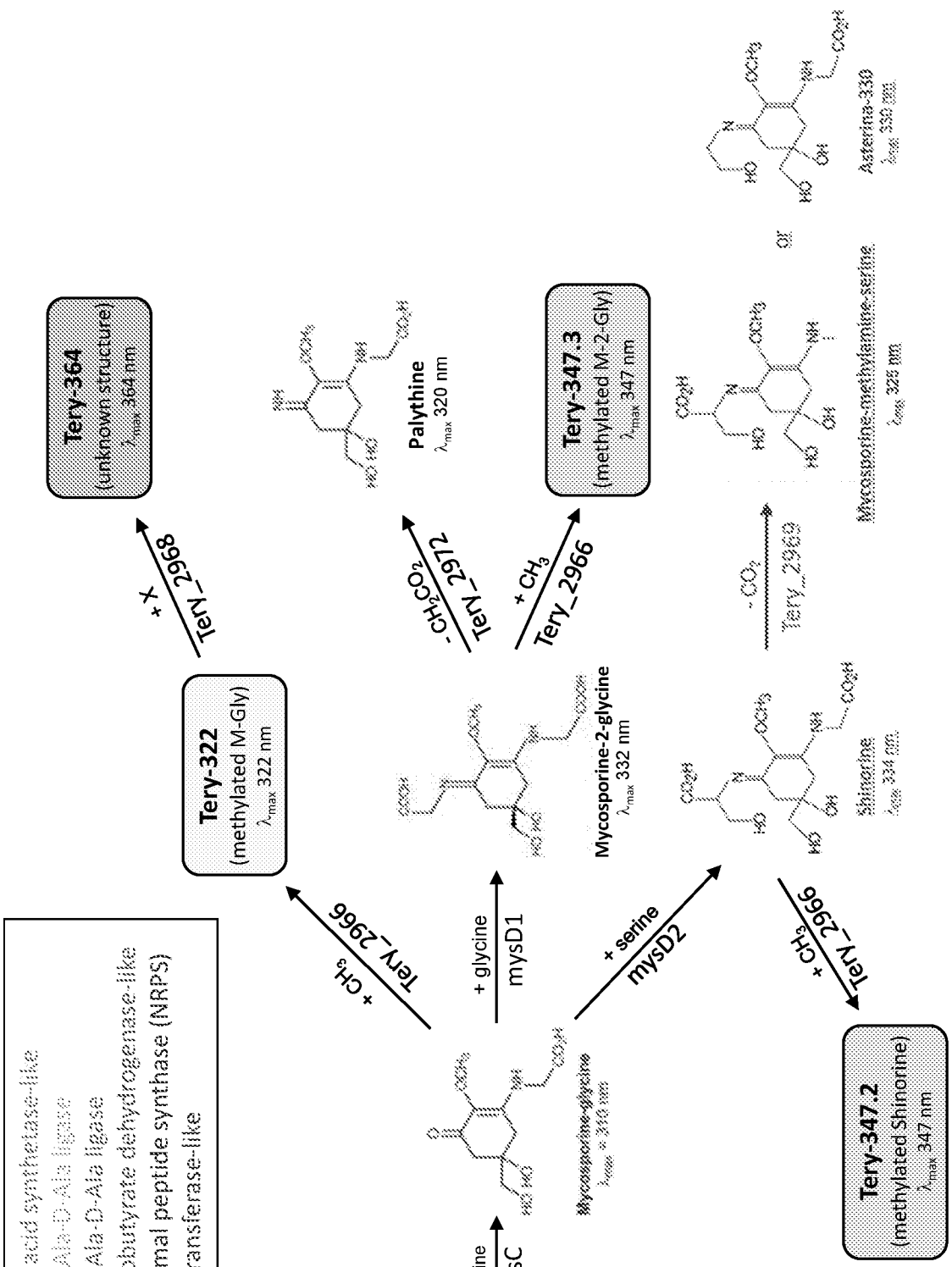

Tery_2972 : Clavaminic acid synthetase-like
Tery_2971 (mysD1) : D-Ala-D-Ala ligase
Tery_2970 (mysD2) : D-Ala-D-Ala ligase
Tery_2969 : Hydroxy-isobutyrate dehydrogenase-like
Tery_2968 : non-ribosomal peptide synthase (NRPS)
Tery_2966 : O-methyl transferase-like
4-Deoxygalactosyl
+ glycine
mysC
Mycosporine-glycine
+ glycine
mysD1
Mycosporine-2-glycine
λmax 332 nm
+ serine
mysD2
Shinorine
λmax 334 nm
Tery-322
(methylated M-Gly)
λmax 322 nm
+ CH3
Tery_2966
Tery-347.2
(methylated Shinorine)
λmax 347 nm
+ CH3
Tery_2966
Tery-347.3
(methylated M-2-Gly)
λmax 347 nm
- CO2
Tery_2969
Mycosporine-methylamine-serine
λmax 328 nm
or
Asterina-330
λmax 330 nm
+ X
Tery_2968
Tery-364
(unknown structure)
λmax 364 nm
- CH2CO2
Tery_2972
Palythine
λmax 320 nm

FIG. 39

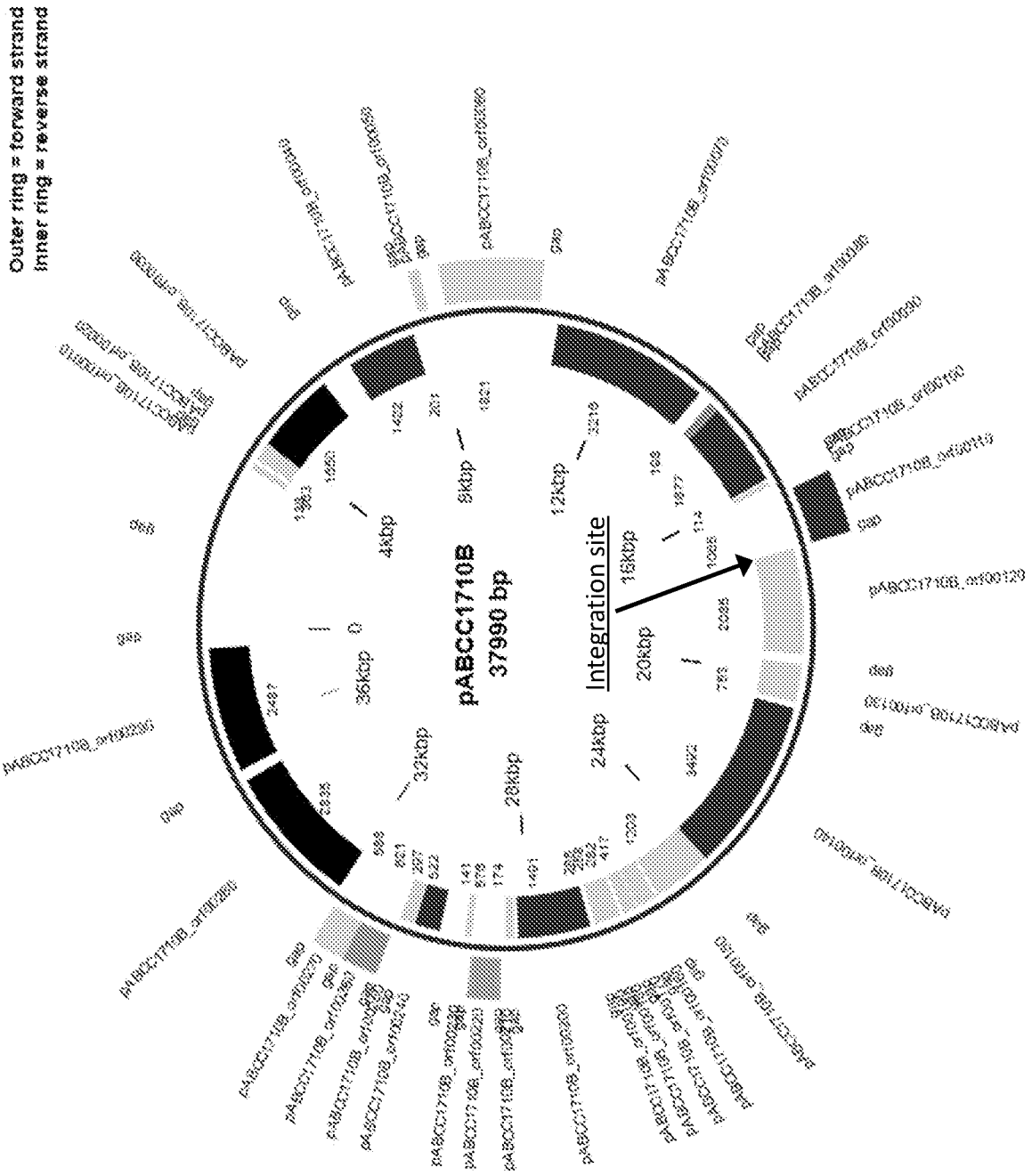

Outer ring = forward strand
Inner ring = reverse strand
pABCC1710B
37990 bp
Integration site
0
4kbp
8kbp
12kbp
16kbp
20kbp
24kbp
28kbp
32kbp
36kbp

FIG. 40

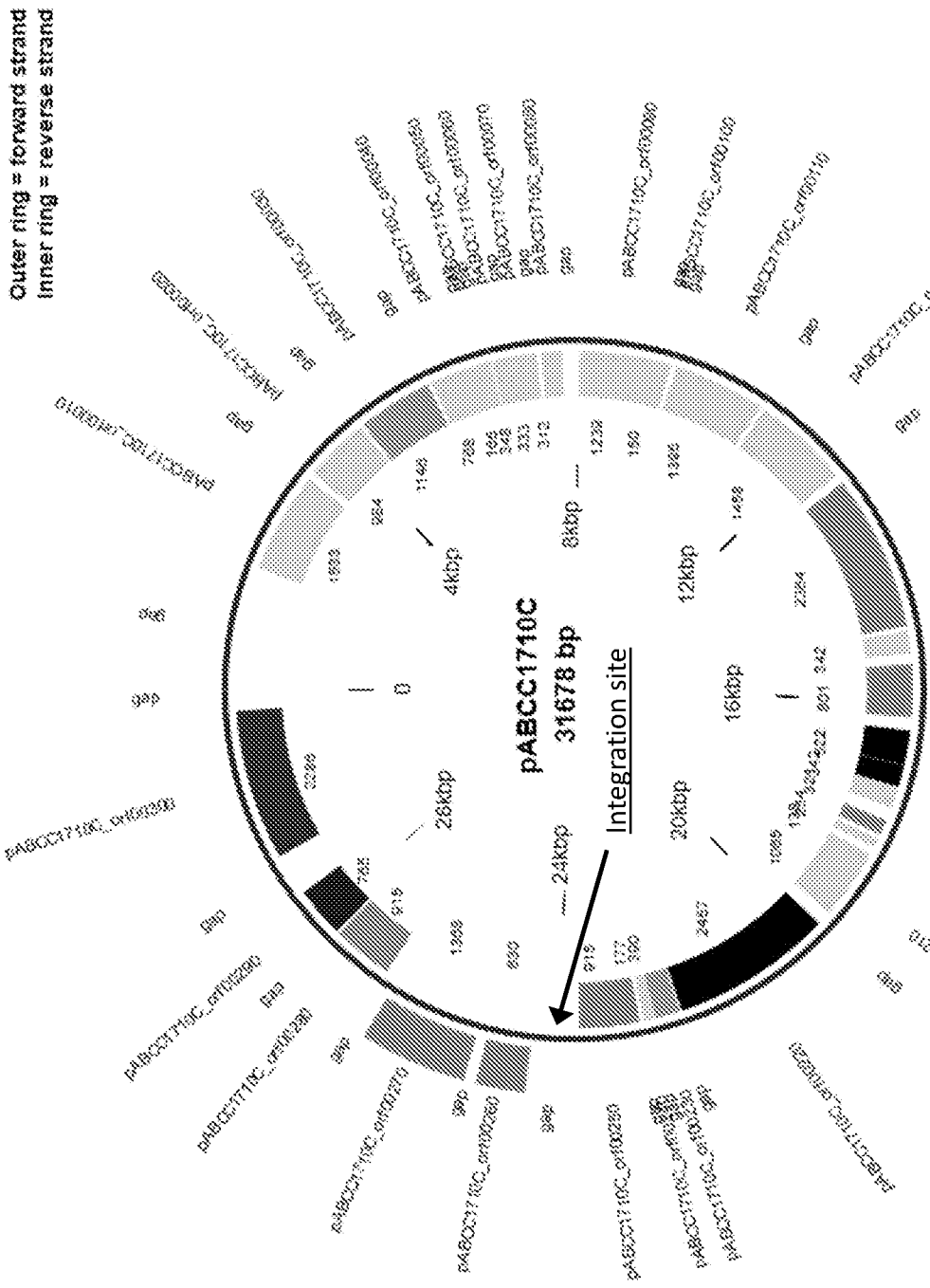

Outer ring = forward strand
Inner ring = reverse strand
pABCC1710C
31678 bp
Integration site
0kbp
4kbp
8kbp
12kbp
16kbp
20kbp
24kbp
28kbp

FIG. 41

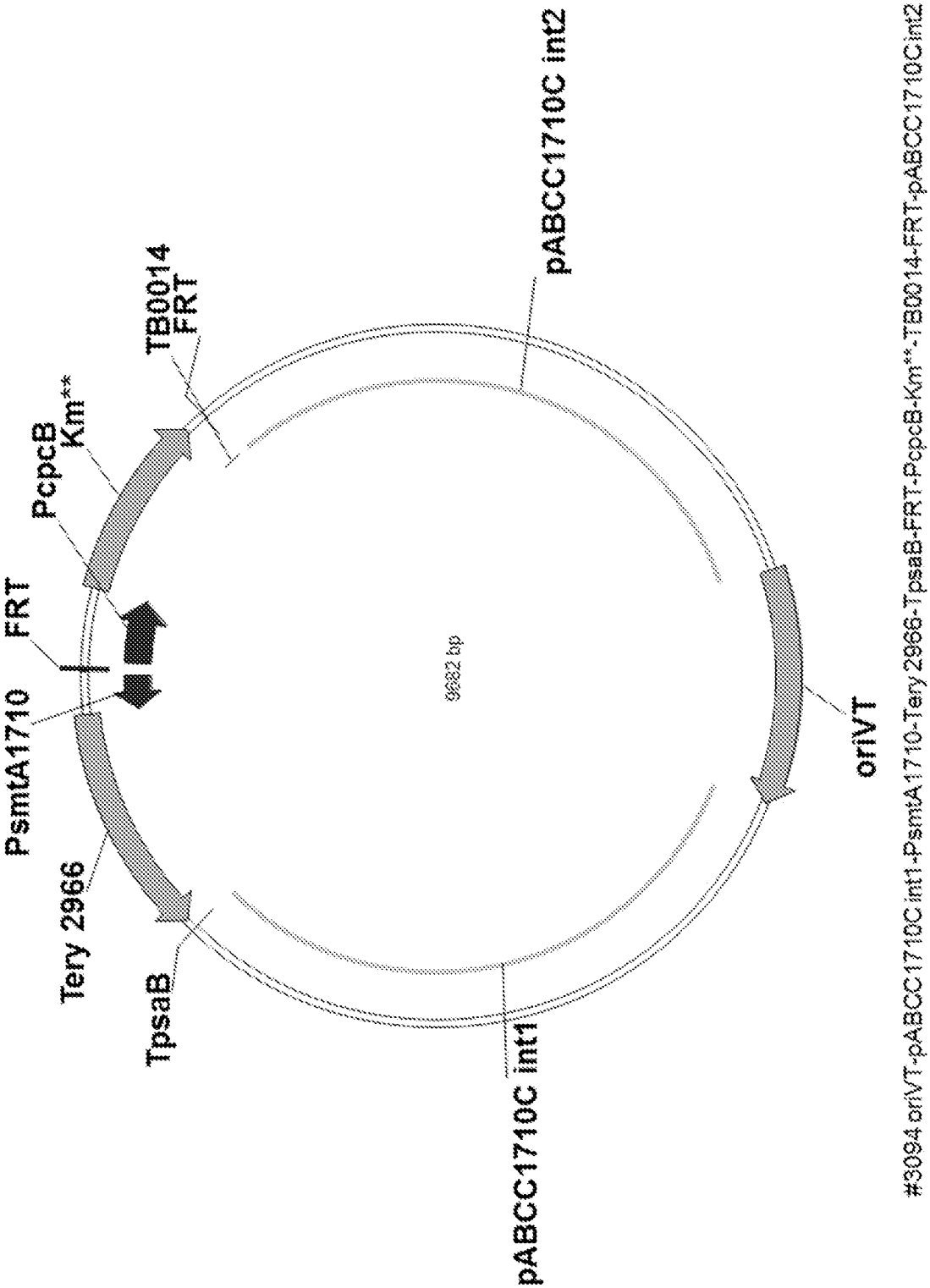

pABCC1710C int2
TB0014 FRT
PcpcB Km**
FRT
PsmtA1710
Tery 2966
TpsaB
pABCC1710C int1
oriVT
9682 bp
#3094 oriVT-pABCC1710C int1-PsmtA1710-Tery 2966-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 42

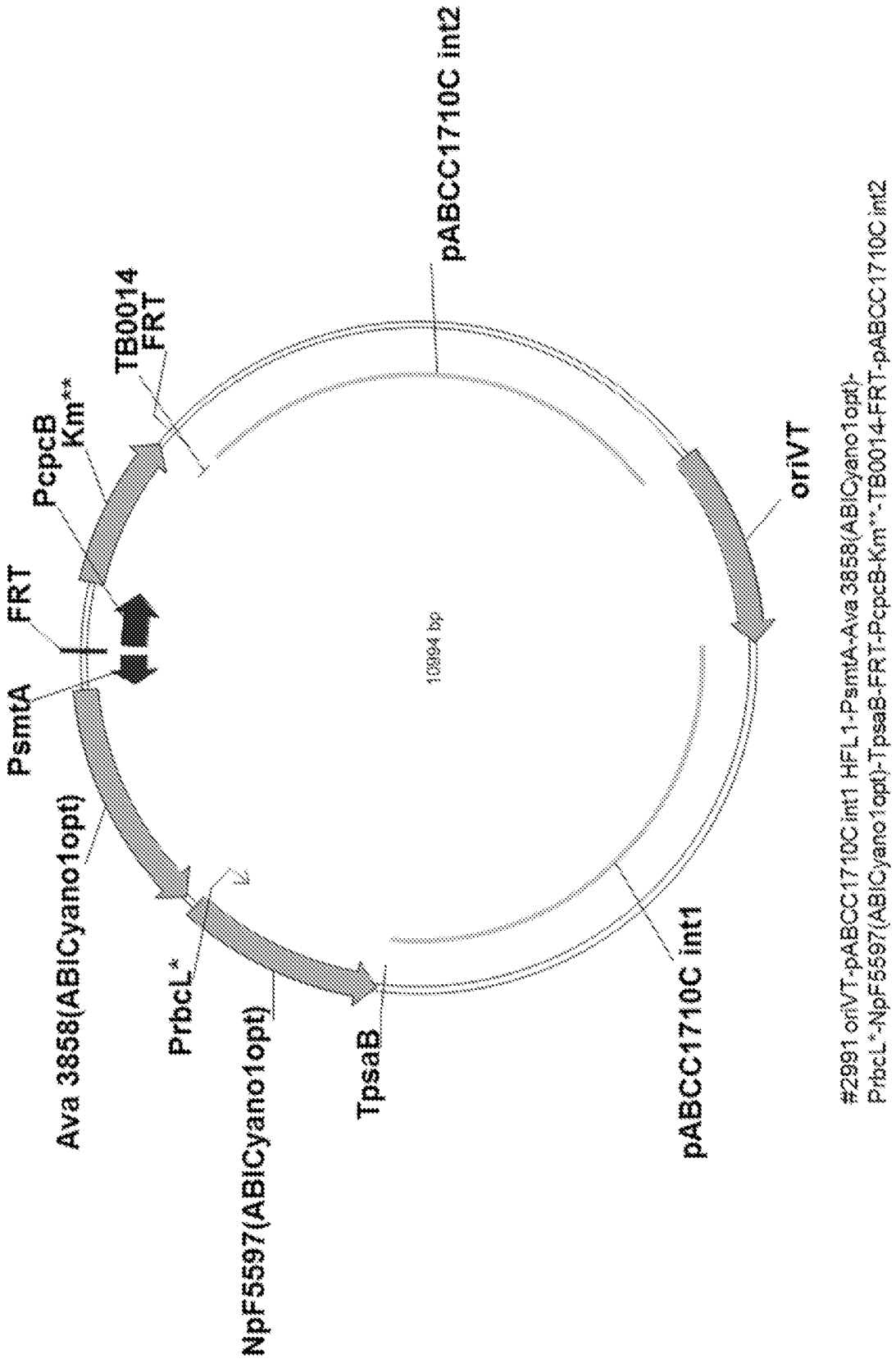

pABCC1710C int2
TB0014 FRT
PcpcB
Km**
FRT
PsmtA
Ava 3858(ABICyano1opt)
PrbcL*
NpF5597(ABICyano1opt)
TpsaB
pABCC1710C int1
oriVT
10894 bp
#2991 oriVT-pABCC1710C int1 HFL1-PsmtA-Ava 3858(ABICyano1opt)-PrbcL*-NpF5597(ABICyano1opt)-TpsaB-FRT-PcpcB-Km**-TB0014-FRT-pABCC1710C int2

FIG. 43

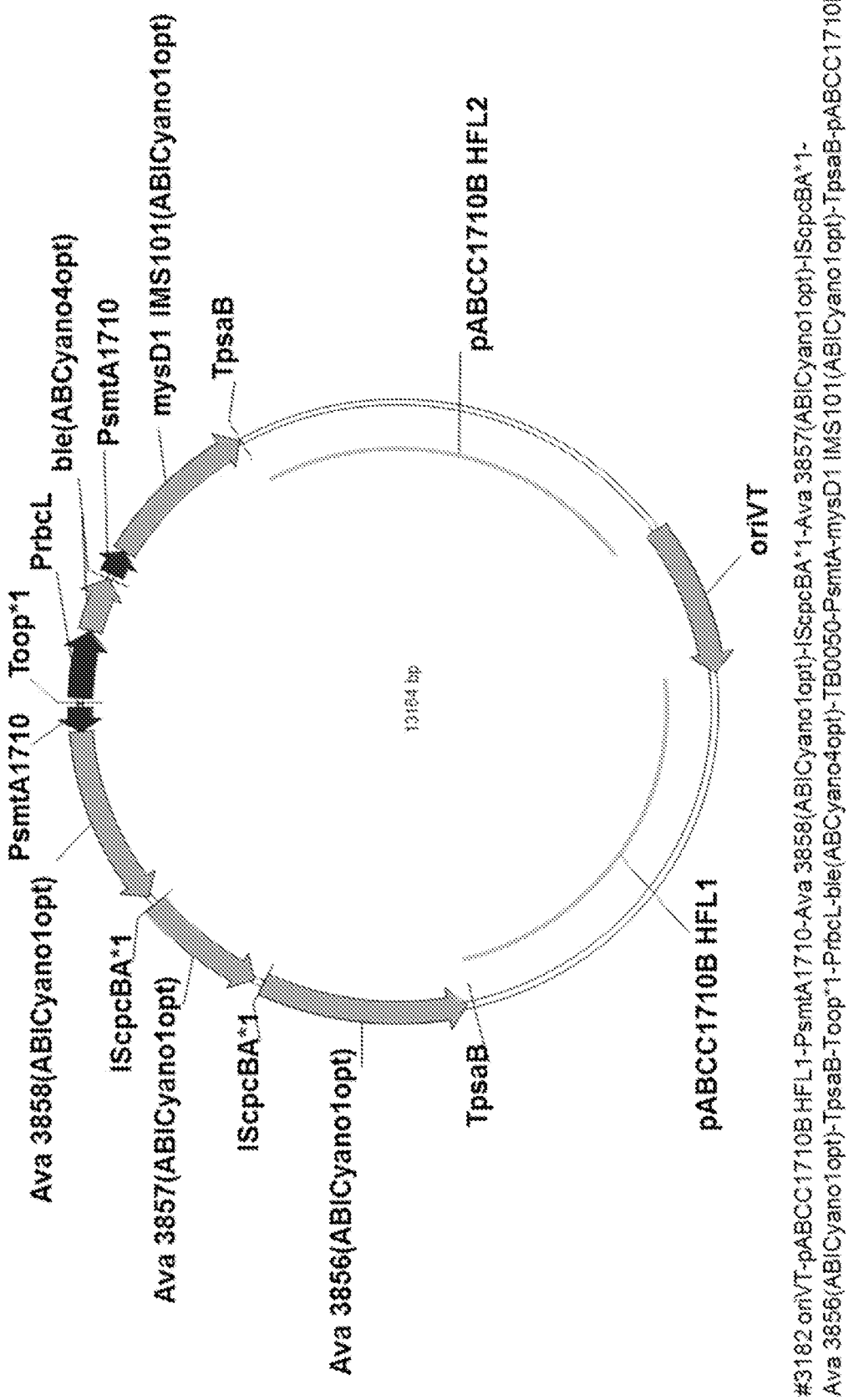

PsmtA1710
Ava 3858(ABICyano1opt)
Toop*1
PrbcL
ble(ABCyano4opt)
PsmtA1710
mysD1 IMS101(ABICyano1opt)
TpsaB
pABCC1710B HFL2
oriVT
pABCC1710B HFL1
TpsaB
Ava 3856(ABICyano1opt)
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
13164 bp
#3182 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-lScpcBA*1-Ava 3857(ABICyano1opt)-lScpcBA*1-
Ava 3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-mysD1 IMS101(ABICyano1opt)-TpsaB-pABCC1710B HFL2

FIG. 44

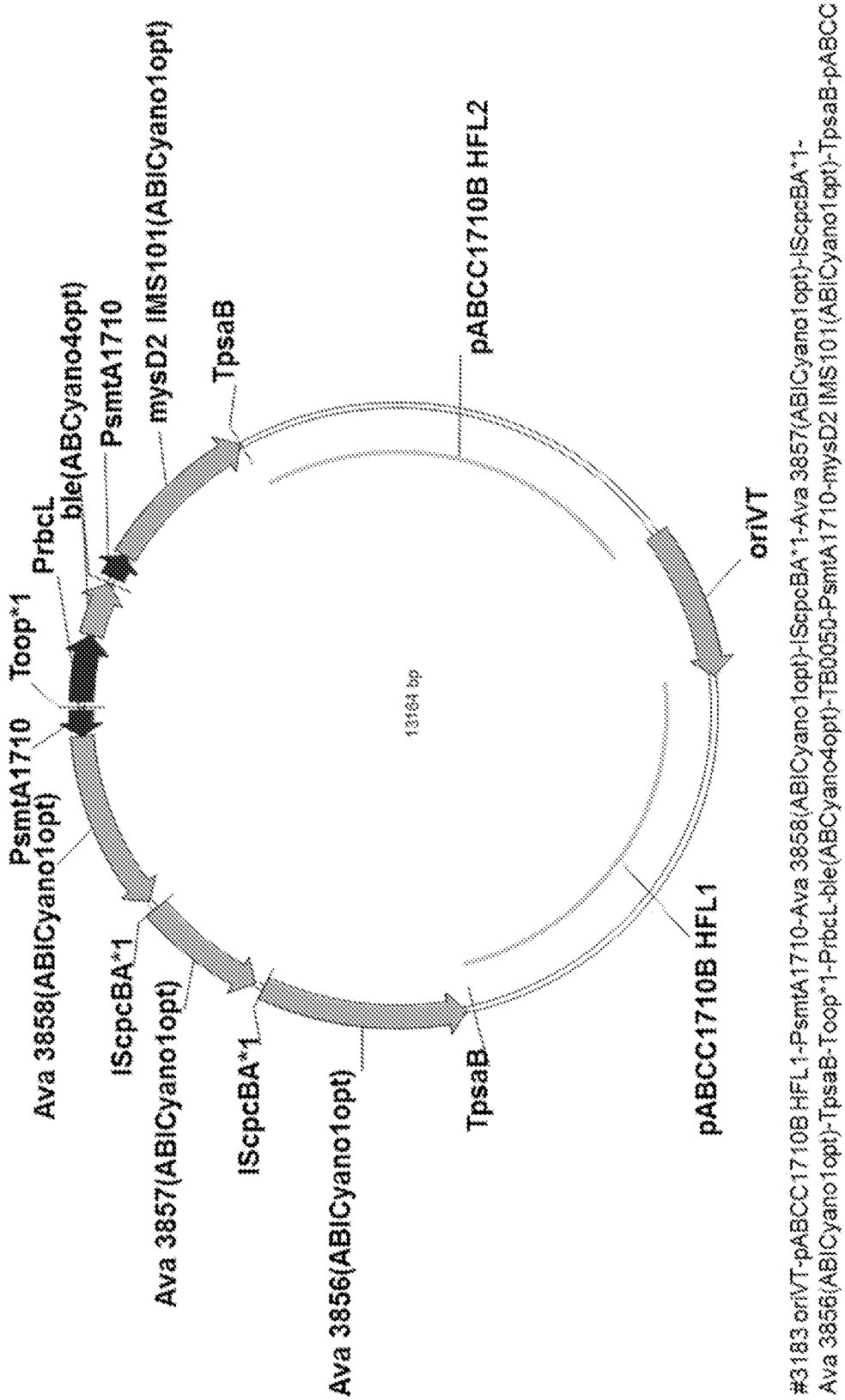

PsmtA1710
Toop*1
PrbcL
ble(ABCyano4opt)
PsmtA1710
mysD2 IMS101(ABICyano1opt)
TpsaB
pABCC1710B HFL2
oriVT
Ava 3858(ABICyano1opt)
IScpcBA*1
Ava 3857(ABICyano1opt)
IScpcBA*1
Ava 3856(ABICyano1opt)
TpsaB
pABCC1710B HFL1
13183 bp
#3183 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-
Ava 3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA1710-mysD2 IMS101(ABICyano1opt)-TpsaB-pABCC1710B HFL2

FIG. 45

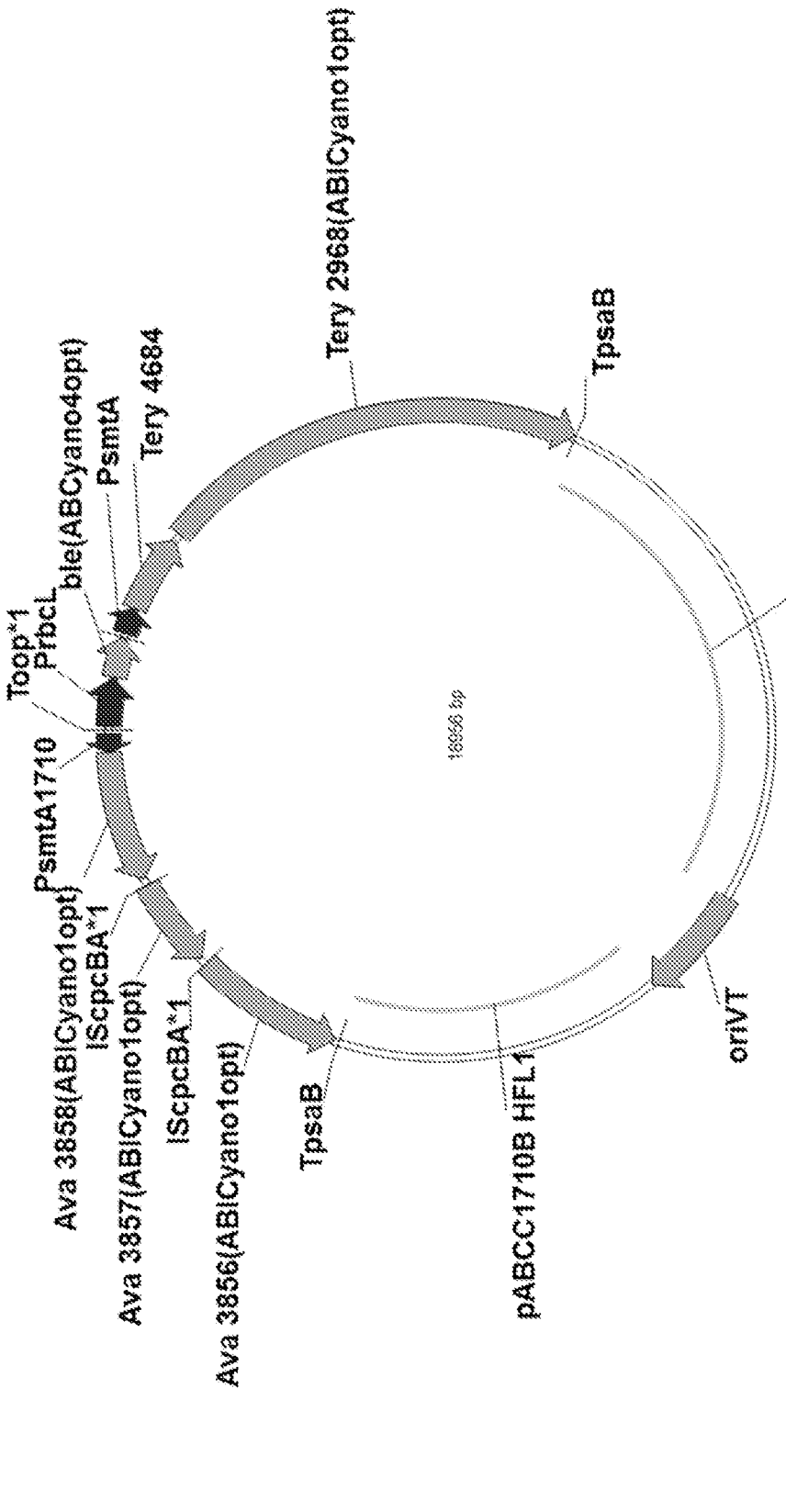
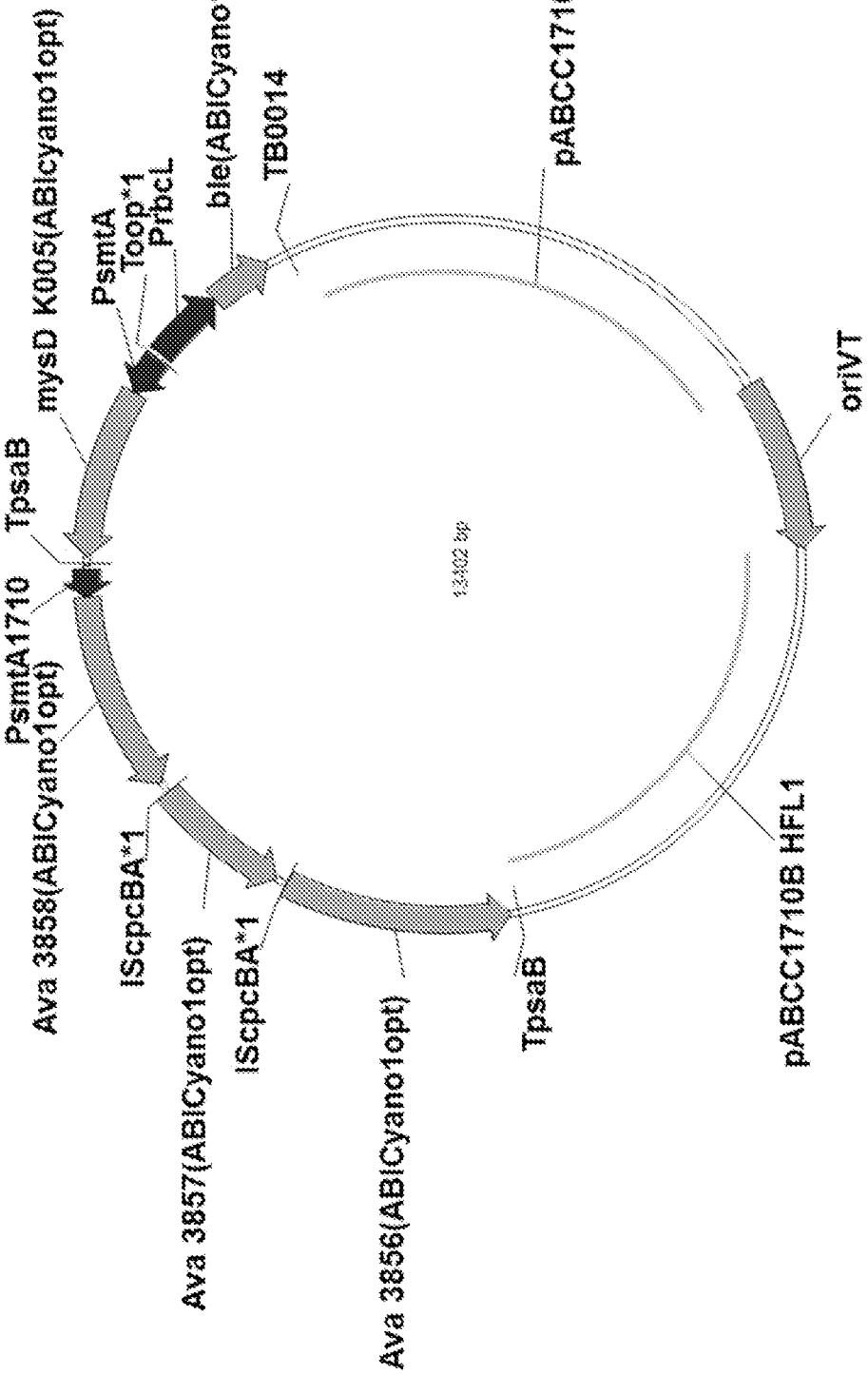
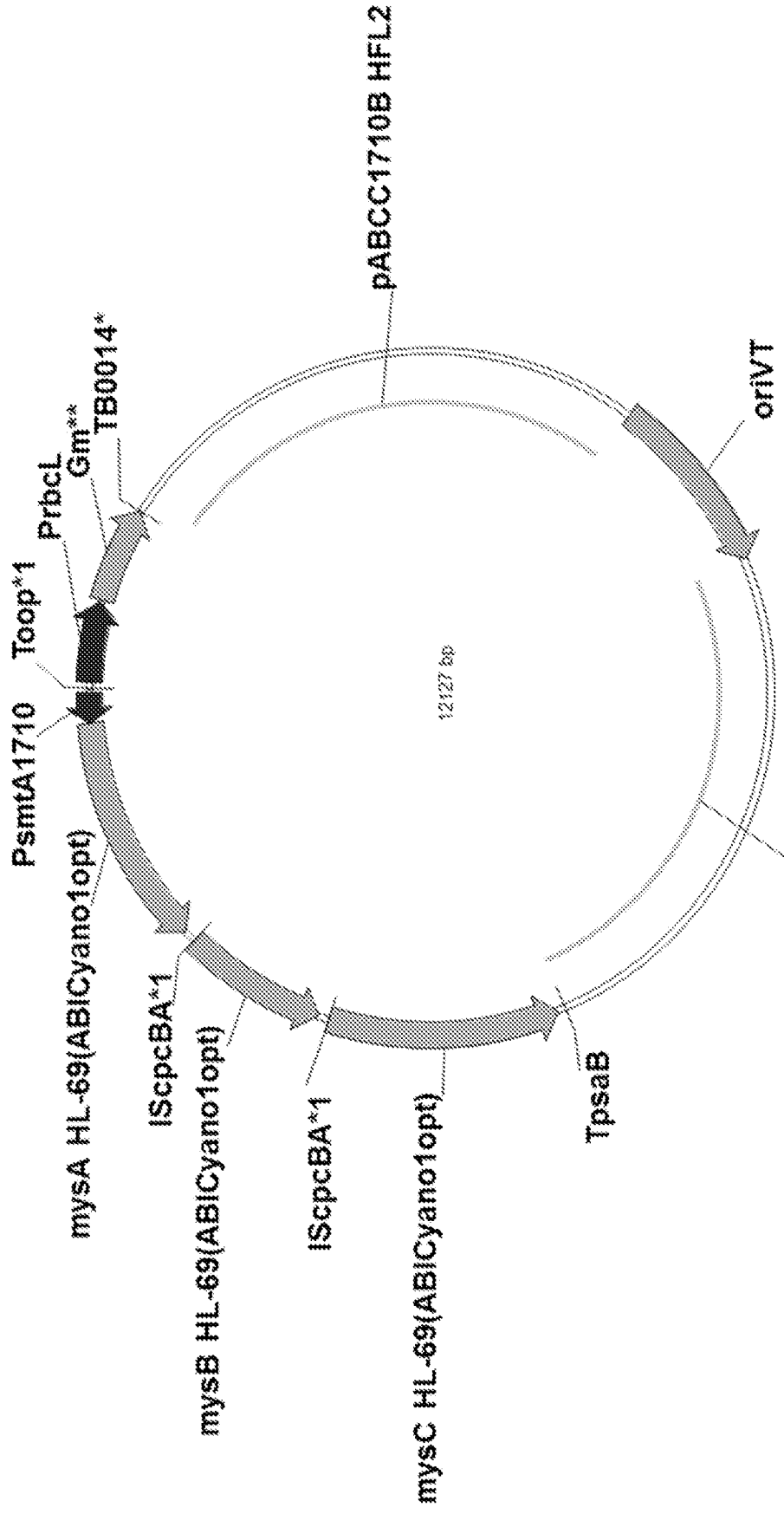

Toop*1
PrbcL
ble(ABCyano4opt)
PsmtA
Tery 4684
Tery 2968(ABICyano1opt)
TpsaB
pABCC1710B HFL3
PsmtA1710
Ava 3858(ABICyano1opt)
lScpcBA*1
Ava 3857(ABICyano1opt)
lScpcBA*1
Ava 3856(ABICyano1opt)
TpsaB
pABCC1710B HFL1
oriVT
16956 bp
#3211 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-lScpcBA*1-Ava 3857(ABICyano1opt)-lScpcBA*1-Ava 3856(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABCyano4opt)-TB0050-PsmtA-Tery 4684-lScpcBA*1-Tery 2968(ABICyano1opt)-TpsaB-pABCC1710B HFL3

FIG. 46



#3287 oriVT-pABCC1710B HFL1-PsmtA1710-Ava 3858(ABICyano1opt)-IScpcBA*1-Ava 3857(ABICyano1opt)-IScpcBA*1-Ava 3856(ABICyano1opt)-TpsaB-PsmtA-mysD K005(ABICyano1opt)-TpsaB-Toop*1-PrbcL-ble(ABICyano1opt)-TB0014-pABCC1710B HFL2

FIG. 47



#3289 oriVT-pABCC1710B_HFL1-PsmtA1710-mysA_HL-69(ABICyano1opt)-lScpcBA*1-mysB_HL-69(ABICyano1opt)-lScpcBA*1-mysC-HL-69(ABICyano1opt)-TpsaB-Toop*1-PrbcL-Gm**-TB0014*-pABCC1710B_HFL2

FIG. 48

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/032485

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/00 C07K14/195 C12P13/04 C12N1/20
ADD.

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)
C12N C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/185018 A1 (NAKED BIOME INC [US]) 26 October 2017 (2017-10-26) whole document esp. seq id nos 4,5,6; paragraph [00280]	1-39
E	WO 2019/094447 A2 (ALGENOL BIOTECH LLC [US]) 16 May 2019 (2019-05-16) whole document esp.seq id nos 3, 6, 9, 164, 166, 168; claim 18 ff	1-39
Y	US 2014/044677 A1 (QVIT-RAZ NOGA [US] ET AL) 13 February 2014 (2014-02-13) whole document esp. seq id nos 4, 5, 6	1-39
Y	US 2017/202762 A1 (IKEDA HARUO [JP] ET AL) 20 July 2017 (2017-07-20) whole document esp. paragraphs [53,67,69,70,86]	1-39

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2019	13/12/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brück, Marianne

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/032485

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	E. P. BALSKUS ET AL: "The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria", SCIENCE, vol. 329, no. 5999, 24 September 2010 (2010-09-24), pages 1653-1656, XP055083282, ISSN: 0036-8075, DOI: 10.1126/science.1193637 whole document esp. pages 1653, 1654	1-39
X	MARIO O. CARIGNAN ET AL: "Characterization of mycosporine-serine-glycine methyl ester, a major mycosporine-like amino acid from dinoflagellates: a mass spectrometry study", JOURNAL OF PHYCOLOGY., vol. 49, no. 4, 1 August 2013 (2013-08-01), pages 680-688, XP055381724, US ISSN: 0022-3646, DOI: 10.1111/jpy.12076	12,13
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Y	whole document esp. page 3	1-39

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/032485

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US 2017202762 A1	20-07-2017	CN 106574282 A	19-04-2017
		EP 3144392 A1	22-03-2017
		JP 5927593 B2	01-06-2016
		JP WO2015174427 A1	20-04-2017
		KR 20170002587 A	06-01-2017
		US 2017202762 A1	20-07-2017
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		WO 2011158041 A2	22-12-2011