

[DESCRIPTION]

[Invention Title]

MICROORGANISM FOR PRODUCING A MYCOSPORINE-LIKE AMINO ACID AND METHOD FOR PRODUCING A MYCOSPORINE-LIKE AMINO ACID USING THE SAME

[Technical Field]

The present disclosure relates to a microorganism for producing a mycosporine-like amino acid, and to a method for producing a mycosporine-like amino acid using the microorganism.

[Background Art]

Ultraviolet radiation emitted from the sun consists of UV-A (Ultraviolet A, which ranges from approximately 320 nm to 400 nm), UV-B (Ultraviolet B, which ranges from approximately 290 nm to 320 nm), and UV-C (Ultraviolet C, which ranges from approximately 100 nm to 280 nm). Among the sun's rays, approximately 6% of the UV-A and UV-B reach the surface of the earth, on the other hand, UV-C does not reach the surface of the earth as it is absorbed and scattered in the earth's ozone layer and the atmosphere.

Although these UV rays may provide beneficial aspects, such as vitamin D synthesis in the body, treatment of skin diseases, sterilization effects, *etc.*, they also impose harmful aspects such as sunburn, skin cancer, aging, photosensitive skin diseases, and mutagenesis. It has been known that UV-A penetrates even to the dermis layer, mainly causing pigmentation and skin aging, and is involved in the development of photosensitive skin diseases, while UV-B is implicated in causing sunburn, pigmentation and skin cancer by penetrating the upper part of the epidermis and dermis layers with high energy rays.

Attempts have been made to block the sunlight in order to prevent these side effects caused by the sun's rays. To this end, types of sunscreen agents can be classified into chemical sunscreen agents and physical sunscreen agents. Chemical sunscreen agents primarily block the penetration of sunlight through absorption, while physical sunscreen agents block the sunlight through reflection and scattering of the sunlight.

Chemical sunscreen agents contain one or more UV-absorbing components, such as PABA, PABA esters (amyl dimethyl PABA, octyl dimethyl PABA), cinnamates (cinoxate),

salicylates (Homomenthyl salicylate), camphor, *etc.* which mainly absorb UV-B, and benzophenone (oxybenzone, dioxybenzone, sulisobenzene), dibenzoyl methane, anthranilate, *etc.*, which mainly absorb UV-A. Although these chemical sunscreen agents may provide a UV-protecting effect by absorbing UV rays, some of which may cause irritation in the skin or the eyes. Particularly, PABA, PABA esters, benzophenones, cinnamates, *etc.*, are known to cause contact dermatitis. Additionally, problems have been reported that some others are associated with developing hypersensitivity reactions in the skin, and thus, the use of chemical sunscreen agents and the amount of their use are restricted in some countries.

Physical sunscreen agents, which contain components that exist in nature, protect the skin by reflecting and scattering UV rays that penetrate the skin. For example, physical sunscreen agents such as titanium dioxide, talc (magnesium silicate), magnesium oxide, zinc oxide, kaolin, *etc.* can provide a UV-protecting effect for both UV-A and UV-B. Further, they have advantages in that they do not have side effects, such as contact dermatitis, and are not easily washed off by water. In contrast, they also have disadvantages in that it may be difficult to maintain an effective amount of physical sunscreen agents while implementing a desired formulation thereof, and they leave a white cast when applied to the skin.

Mycosporine-like amino acids (MAAs) are substances that are found in natural organisms and are known to effectively absorb UVA (320 nm to 400 nm) and UVB (290 nm to 320 nm). There are 35 species or more of MAAs in nature according to the type of amino acids, cyclohexenone or cyclohexenimine ring, which serve as precursors. (*Mar. Biol.*, 1991, 108: 157-166; *Planta Med.*, 2015, 81: 813-820). Recently, various glycosylated MAAs are found in microalgae, which have been reported to play a role as excellent antioxidants (*Journal of Photochemistry and Photobiology*, 2015, 142: 154-168). Additionally, MAAs are known to not only provide UV protection but also show resistance to oxidation, osmosis, and thermal stress (*Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 2007, 146: 60-78; *J. Photochem. Photobiol. B.*, 2007, 89: 29-35).

However, the amount of MAAs produced in microalgae is very small at a few μg level, and the conditions for separating, extracting and purifying MAAs by culturing microalgae are complicated, and thus, it would be difficult to produce MAAs in a large-scale production.

[Prior Art Literature]

[Non-Patent Literature]

- (Non-Patent Literature 1) Comp. Biochem. Physiol. B 1995, 112: 105-114.
- (Non-Patent Literature 2) FEMS Microbiol Lett. 2007, 269: 1-10.
- (Non-Patent Literature 3) Ann. Rev. Physiol. 2002, 64: 223-262.
- (Non-Patent Literature 4) Mar. Biol. 1991, 108: 157-166.
- (Non-Patent Literature 5) Journal of Photochemistry and Photobiology B: Biology. 2015, 142: 154-168
- (Non-Patent Literature 6) Biol. Rev. 1999, 74: 311-345.
- (Non-Patent Literature 7) Mol. Biol. Evol. 2006, 23: 1437-1443.
- (Non-Patent Literature 8) Science, 2010, 329: 1653-1656.
- (Non-Patent Literature 9) Genomics 2010, 95: 120-128.
- (Non-Patent Literature 10) Geomicrobiol. J. 1997. 14: 231-241.
- (Non-Patent Literature 11) Comp. Biochem. Physiol. C Toxicol. Pharmacol. 2007. 146: 60-78.
- (Non-Patent Literature 12) Can. J. Bot. 2003. 81: 131-138.
- (Non-Patent Literature 13) J. Photochem. Photobiol. B. 2007, 89: 29-35.
- (Non-Patent Literature 14) J. Bacteriol. 2011. 193(21): 5923-5928.
- (Non-Patent Literature 15) Planta Med. 2015. 81: 813-820
- (Non-Patent Literature 16) ACS Appl. Mater. Interfaces. 2015. 7: 16558-16564
- (Non-Patent Literature 17) Appl Environ Microbiol. 2016, 82(20): 6167-6173
- (Non-Patent Literature 18) ChemBioChem. 2015, 16: 320-327
- (Non-Patent Literature 19) Methods Mol Biol. 2013, 1073: 43-7
- (Non-Patent Literature 20) Enzyme Microb Technol., 2016, Jan, 82: 96-104
- (Non-Patent Literature 21) Nature Review, 2011, 9: 791-802

[Disclosure]

[Technical Problem]

The present inventors have made extensive efforts to increase the production of MAAs in microorganisms. As a result, it was confirmed that the production of MAAs was increased through various studies on the inactivation of 3-dehydroquinase dehydratase activity in the microorganism for producing MAAs, thereby completing the present disclosure.

[Technical Solution]

It is one object of the present disclosure to provide a microorganism for producing a mycosporine-like amino acid, wherein an activity of 3-dehydroquinate dehydratase is inactivated as compared to a non-modified microorganism.

It is another object of the present disclosure to provide a method for producing a mycosporine-like amino acid, including:

culturing the microorganism; and

recovering a mycosporine-like amino acid from the cultured microorganism or medium.

[Advantageous Effects]

The microorganism of the present disclosure shows an improved ability for producing a mycosporine-like amino acid, and thus can be effectively used in the production of a mycosporine-like amino acid.

[Detailed Description of Preferred Embodiments]

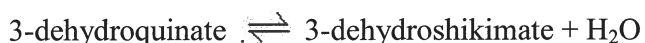
The specific details of the present disclosure may be explained as follows.

Meanwhile, the explanations and embodiments disclosed in the present disclosure may be applied to other explanations and embodiments, respectively. That is, all combinations of various elements disclosed herein belong to the scope of the present disclosure. Additionally, the scope of the present disclosure should not be limited by the specific descriptions described hereinbelow. Additionally, those of ordinary skill in the art may be able to recognize or confirm, using only conventional experimentation, many equivalents to the particular aspects of the invention described in this application. Furthermore, it is also intended that these equivalents be included in the present disclosure.

In order to achieve the objects above, an aspect of the present disclosure provides a microorganism for producing a mycosporine-like amino acid, wherein the activity of 3-dehydroquinate dehydratase is inactivated as compared to a non-modified microorganism.

As used herein, the term “3-dehydroquinate dehydratase” refers to an enzyme that catalyzes the reversible reaction represented by Reaction Scheme below, and may specifically convert 3-dehydroquinate into 3-dehydroshikimate, but is not limited thereto.

[Reaction Scheme]



As used herein, the term “inactivation” refers to a case where the activity of an enzymatic protein originally possessed by a microorganism is weakened compared to the endogenous activity thereof or the activity before modification; a case where the protein is not in any way expressed; or a case where the protein is expressed but exhibits no activity. The inactivation is a concept that includes a case where the activity of an enzyme itself is weakened or eliminated compared to the activity of the enzyme originally possessed by a microorganism, due to a modification in the polynucleotide encoding the enzyme, *etc.*; a case where the level of overall enzyme activity within a cell is reduced or eliminated compared to the wild-type microorganism, due to inhibition of expression of the gene encoding the enzyme, or inhibition of translation, *etc.*; a case where a part or the entirety of the gene encoding the enzyme is deleted; and a combination thereof, but is not limited thereto. The term “non-modified microorganism” refers to a microorganism having the activity of a specific protein originally possessed by the parental strain prior to modification thereof, when the traits of the microorganism are modified through genetic modification in the specific protein possessed by a microorganism for comparison due to natural or artificial factors. As used herein, the “non-modified microorganism” can be interchangeably used with “a microorganism having an endogenous activity”.

The inactivation of the enzymatic activity may be achieved by various methods well known in the art. Examples of the methods include 1) a method of deleting a part or the entirety of a gene encoding the enzyme on a chromosome; 2) a method of modifying an expression regulatory sequence such that the expression of the gene encoding the protein on the chromosome is reduced; 3) a method of modifying a gene sequence encoding the protein on the chromosome such that the activity of the protein is removed or weakened; 4) a method of introducing an antisense oligonucleotide that binds complementarily to the transcript of the gene encoding the protein on the chromosome (*e.g.*, antisense RNA); 5) a method of artificially adding a sequence complementary to the Shine-Dalgarno sequence of the gene encoding the protein on the chromosome to the upstream of the Shine-Dalgarno sequence to form a secondary structure, thereby making the adhesion of ribosome impossible; and 6) a method of reverse

transcription engineering (RTE), which adds a promoter, which is to be reverse-transcribed, to the 3' end of the open reading frame (ORF) of the polynucleotide sequence encoding the protein, or a combination thereof, but are not limited particularly thereto.

The method of deleting a part or the entirety of a gene encoding the protein on the chromosome may be performed by replacing the polynucleotide encoding the endogenous target protein within the chromosome with a polynucleotide having a partially deleted nucleic acid sequence, or a marker gene through a vector for chromosomal insertion into a microorganism. As an example of the method of deleting a part or the entirety of the polynucleotide, a method of deleting a polynucleotide by homologous recombination may be used, but is not limited thereto.

The method of modifying an expression regulatory sequence may be performed by inducing a modification in the expression regulatory sequence through deletion, insertion, conservative or non-conservative substitution, or a combination thereof so as to further weaken the activity of the expression regulatory sequence; or by replacing the sequence with a nucleic acid sequence having a weaker activity. The expression regulatory sequence may include a promoter, an operator sequence, a sequence encoding a ribosome-binding domain, and a sequence for regulating the termination of transcription and translation, but is not limited thereto.

The method of modifying the gene sequence on the chromosome may be performed by inducing a modification in the gene sequence through deletion, insertion, conservative or non-conservative substitution, or a combination thereof so as to further weaken the activity of the enzyme; or by replacing the sequence with a gene sequence modified to have a weaker activity or a gene sequence modified to have no activity at all, but is not limited thereto.

The polynucleotide may be described as a gene in cases where it refers to an assemblage of polynucleotides capable of carrying out functions. In the present disclosure, polynucleotides and genes may be used interchangeably, and polynucleotide sequences and nucleotide sequences may be used interchangeably.

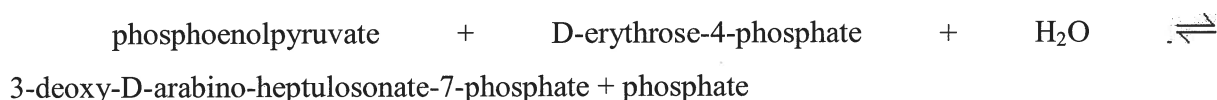
As used herein, the term "part", although it may vary depending on the kinds of polynucleotide, may specifically refer to 1 to 300 nucleotides, more specifically 1 to 100 nucleotides, and even more specifically 1 to 50 nucleotides, but is not particularly limited thereto.

In the microorganism of the present disclosure, an activity of at least one selected from the group consisting of 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate

synthetase, transketolase I/II, and 3-dehydroquinase synthase, specifically the activity of one or more, two or more, three or more, or all enzymes may be enhanced as compared to a non-modified microorganism.

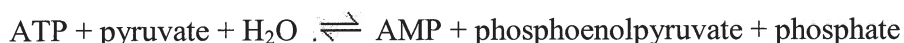
The 2-dehydro-3-deoxyphosphoheptonate aldolase refers to an enzyme that catalyzes the reversible reaction represented by Reaction Scheme below, and may specifically synthesize 3-deoxy-arabino-heptulosonate 7-phosphate, but is not limited thereto.

[Reaction Scheme]



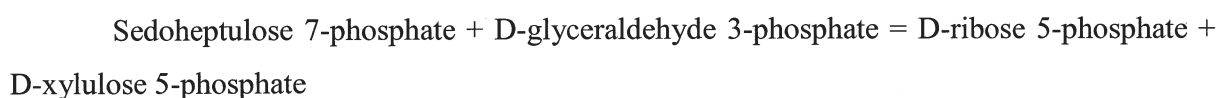
The phosphoenolpyruvate synthetase refers to an enzyme that catalyzes the reversible reaction represented by Reaction Scheme below, and may specifically synthesize phosphoenolpyruvate, but is not limited thereto.

[Reaction Scheme]



The transketolase I/II refers to an enzyme that catalyzes the reversible reaction represented by Reaction Scheme below.

[Reaction Scheme]



The 3-dehydroquinase synthase refers to an enzyme that catalyzes the reversible reaction represented by Reaction Scheme below, and may specifically synthesize 3-dehydroquinase (3-DHQ), but is not limited thereto.

[Reaction Scheme]



As used herein, the term “enhancement of activity” means that the activity of an enzymatic protein is introduced, or the activity is enhanced as compared to the endogenous activity possessed by a microorganism or the activity before modification. The “introduction”

of the activity means that the activity of a specific polypeptide that the microorganism did not originally have is naturally or artificially expressed. For example, the enhancement of the activity may include both an enhancement by introducing 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase I/II, and/or 3-dehydroquinate synthase; or an enhancement of the endogenous activity of 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase I/II, and/or 3-dehydroquinate synthase. Specifically, the enhancement of the activity in the present disclosure may be performed by the following methods:

- 1) a method of increasing the copy number of the polynucleotide encoding the enzymes;
- 2) a method of modifying an expression regulatory sequence such that the expression of the polynucleotide is increased;
- 3) a method of modifying the polynucleotide sequence on the chromosome such that the activity of the enzymes is enhanced; and
- 4) a method of modification to enhance the activity by a combination of the above methods, but is not limited thereto.

The increasing of the copy number of the polynucleotide in method 1) above may be performed in a form in which the polynucleotide is operably linked to a vector, or by inserting into a chromosome of a host cell, but is not particularly limited thereto. Additionally, in an aspect to increase the copy number, it may be performed by introducing into a host cell a foreign polynucleotide that exhibits the activity of the enzyme, or a modified polynucleotide in which the codons of the foreign polynucleotide have been optimized. The foreign polynucleotide may be used without limitation to its origin or sequence as long as it exhibits an activity identical or similar to that of the enzyme. The introduction may be performed by those skilled in the art by selecting a suitable transformation method known in the art, and an enzyme may be produced as the introduced polynucleotides are expressed in the host cell, thereby increasing its activity.

Next, the modification of an expression regulatory sequence such that the expression of the polynucleotide is increased in method 2) may be performed by inducing a modification in the sequence through deletion, insertion, or non-conservative or conservative substitution of a nucleic acid sequence, or a combination thereof so as to further enhance the activity of the expression regulatory sequence, or by replacing with a nucleic acid sequence having a stronger activity, but is not particularly limited thereto. Additionally, the expression regulatory sequence may include a promoter, an operator sequence, a sequence encoding a

ribosome-binding domain, a sequence regulating the termination of transcription and translation, *etc.*, but is not particularly limited thereto.

Specifically, a strong heterologous promoter may be linked to the upstream region of the expression unit of the polynucleotide instead of the original promoter. Examples of the strong promoter include *CJ7* promoter, *lysCPI* promoter, *EF-Tu* promoter, *groEL* promoter, *aceA* or *aceB* promoter, *etc.* More specifically, the expression rate of the polynucleotide encoding the enzyme may be enhanced by operably linking the polynucleotide with the *lysCPI* promoter (WO2009/096689) or *CJ7* promoter (WO2006/065095), but is not limited thereto.

Further, the modification of the polynucleotide sequence on the chromosome in method 3) may be performed by inducing a modification in the expression regulatory sequence through deletion, insertion, or non-conservative or conservative substitution of a nucleic acid sequence, or a combination thereof so as to further enhance the activity of the polynucleotide sequence, or by replacing the polynucleotide sequence with a polynucleotide sequence modified to have a stronger activity, but is not particularly limited thereto.

Finally, the method of modification to enhance the activity by a combination of methods 1) to 3) in method 4) may be performed by a combined application of at least one of the following methods: increasing of the copy number of the polynucleotide encoding the protein; modification of an expression regulatory sequence such that the expression of the polynucleotide is increased; modification of the polynucleotide sequence on the chromosome, and modification of a foreign polynucleotide exhibiting the activity of the enzyme or a codon-optimized modified polynucleotide thereof.

As used herein, the term “vector” refers to a DNA construct containing the nucleotide sequence of a polynucleotide encoding the target protein, which is operably linked to a suitable regulatory sequence such that the target protein can be expressed in an appropriate host. The regulatory sequence includes a promoter capable of initiating transcription, any operator sequence for controlling the transcription, a sequence encoding an appropriate mRNA ribosome-binding domain, and a sequence regulating the termination of transcription and translation. After being transformed into a suitable host cell, the vector may be replicated or function irrespective of the host genome, and may be integrated into the host genome itself.

The vector used in the present disclosure is not particularly limited as long as it can be replicated in a host cell, and any vector known in the art may be used. Examples of conventionally used vectors may include natural or recombinant plasmids, cosmids, viruses, and

bacteriophages. For example, as a phage vector or cosmid vector, pWE15, M13, MBL3, MBL4, IXII, ASHII, APII, t10, t11, Charon4A, Charon21A, *etc.*, may be used, and as a plasmid vector, those based on pBR, pUC, pBluescriptII, pGEM, pTZ, pCL, pET, *etc.* may be used. Specifically, the vectors pDZ, pACYC177, pACYC184, pCL, pECCG117, pUC19, pBR322, pMW118, pCC1BAC, *etc.* may be used, but the vector is not limited thereto.

The vector that can be used in the present disclosure is not particularly limited, and a known expression vector may be used. Additionally, a polynucleotide encoding a target protein may be inserted into the chromosome through a vector for chromosomal insertion. The insertion of the polynucleotide into the chromosome may be performed using any method known in the art, for example, by homologous recombination, but is not limited thereto. A selection marker for confirming the insertion into the chromosome may be further included. The selection marker is used for selecting a cell transformed into a vector, *i.e.*, in order to confirm whether the target nucleic acid molecules have been inserted, and markers that provide selectable phenotypes, such as drug resistance, nutrient requirement, resistance to cytotoxic agents, or expression of surface proteins, may be used. Only the cells expressing the selection markers can survive or express other phenotypic traits under the environment treated with selective agents, and thus, the transformed cells can be easily selected.

As used herein, the term “transformation” refers to a process of introducing a vector including a polynucleotide encoding a target polypeptide into a host cell, thereby enabling the expression of the protein encoded by the polynucleotide in the host cell. As long as the transformed polynucleotide can be expressed in the host cell, it does not matter whether it is inserted into the chromosome of a host cell and located therein, or located outside the chromosome, and both cases may be included. Additionally, the polynucleotide includes DNA and RNA which encode the target polypeptide. The polynucleotide may be introduced in any form as long as it can be introduced into a host cell and expressed therein. For example, the polynucleotide may be introduced into a host cell in the form of an expression cassette, which is a gene construct including all elements necessary for self-expression. The expression cassette may conventionally include a promoter operably linked to the polynucleotide, a terminator, a ribosome-binding domain, and a stop codon. The expression cassette may be in the form of an expression vector capable of self-replication. Additionally, the polynucleotide may be introduced into a host cell as it is and operably linked to a sequence necessary for its expression

in the host cell, but is not limited thereto. The method for transforming the vector includes any method of introducing a nucleic acid into a cell, and may be performed by selecting a suitable standard technique known in the art according to the host cell. For example, the transformation may be carried out via electroporation, calcium phosphate (CaPO_4) precipitation, calcium chloride (CaCl_2) precipitation, microinjection, a polyethylene glycol (PEG) technique, a DEAE-dextran technique, a cationic liposome technique, a lithium acetate-DMSO technique, *etc.*, but the method is not limited thereto.

Further, as used above, the term “operably linked” refers to a functional linkage between the above polynucleotide sequence and a promoter sequence which initiates and mediates the transcription of the polynucleotide encoding the target protein of the present disclosure. The operable linkage may be prepared using a genetic recombinant technique known in the art, and site-specific DNA cleavage and ligation may be performed using enzymes such as lysases, ligases, *etc.* known in the art, but is not limited thereto.

The genetic information of 3-dehydroquinate dehydratase, 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase can be obtained from a known database, and examples thereof include GenBank of National Center for Biotechnology Information (NCBI), *etc.*, but the known database is not limited thereto.

The 3-dehydroquinate dehydratase, 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase may not be limited by their origins or sequences, since there may be a difference in the amino acid sequence of the protein that exhibits the activity depending on the microbial species or microorganisms.

Specifically, the 3-dehydroquinate dehydratase may be a protein including an amino acid sequence of SEQ ID NO: 72 or 80, and 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase may be a protein including an amino acid sequence of SEQ ID NOS: 74, 76, 78, and 84, respectively, but are not limited thereto. In the present disclosure, “the protein including an amino acid sequence” may be used interchangeably with the expression “the protein having an amino acid sequence” or “the protein consisting of an amino acid sequence”.

Additionally, in the present disclosure, the enzymes may not only include the protein having an amino acid sequence of SEQ ID NO: described above, but also a protein having a

homology of 80% or more, specifically 90% or more, more specifically 95% or more, even more specifically 99% or more to the above amino acid sequences, as long as the protein has a biological activity identical or corresponding to that of each enzyme.

Further, it is apparent that, as an amino acid sequence having a homology to the above sequences, an amino acid sequence with deletion, modification, substitution, or addition of a part of the sequence also falls within the scope of the present disclosure as long as the amino acid sequence has a biological activity substantially identical or corresponding to that of the enzymatic protein having an amino acid sequence of SEQ ID NO: described above.

The polynucleotide encoding 3-dehydroquinate dehydratase, 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase of the present disclosure may include a polynucleotide having an amino acid sequence of SEQ ID NO: described above, or a polynucleotide encoding a protein having a homology of 80% or more, specifically 90% or more, more specifically 95% or more, even more specifically 99% or more to the amino acid sequences, as long as it has a biological activity identical or corresponding to that of each enzyme.

Additionally, as for the polynucleotide encoding 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase, considering the codons preferred by organisms to express the protein due to codon degeneracy, various modifications may be executed on the coding region within the scope without changing the amino acid sequence of the polypeptide. Accordingly, the polynucleotide may include any polynucleotide sequence encoding each enzymatic protein without limitation.

Further, a probe which can be prepared from a known gene sequence, for example, any sequence which hybridizes with a sequence complementary to all or a part of the nucleotide sequence under stringent conditions to encode a protein having the activity of 3-dehydroquinate dehydratase, 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase, and 3-dehydroquinate synthase may be included without limitation.

As used herein, the term "homology" refers to the degree of correspondence to a given amino acid sequence or nucleotide sequence, and may be expressed as a percentage. In the present specification, a homologous sequence having an activity which is identical or similar to that of the given amino acid sequence or nucleotide sequence may be indicated in terms of "% homology". For example, the homology may be confirmed using a standard software for calculating parameters such as score, identity, and similarity, specifically, BLAST 2.0, or by

comparing sequences via hybridization experiments under defined stringent conditions, and the defined appropriate hybridization conditions are within the skill of the art, and may be determined by a method well known to those skilled in the art (For example, J. Sambrook et al., *Molecular Cloning, A Laboratory Manual, 2nd Edition*, Cold Spring Harbor Laboratory press, Cold Spring Harbor, New York, 1989; F.M. Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York). The term “stringent conditions” refer to the conditions which allow the specific hybridization between the polynucleotides. Such conditions are specifically disclosed in the literature (e.g., J. Sambrook et al.).

As used herein, the term “mycosporine-like amino acids (MAAs)” refer to cyclic compounds that absorb ultraviolet rays. In the present disclosure, any mycosporine-like amino acid may be included without limitation as long as it can absorb UV rays, but it may specifically be a compound containing a central cyclohexenone or cyclohexenimine ring; or a compound in which various substances, such as amino acid, *etc.*, are bound to the central ring. More specifically, it may be mycosporine-2-glycine, palythanol, palythenic acid, deoxygadusol, mycosporine-methylamine-threonine, mycosporine-glycine-valine, palythine, asterina-330, shinorine, porphyra-334, euhalothece-362, mycosporine-glycine, mycosporine-ornithine, mycosporine-lysine, mycosporine-glutamic acid-glycine, mycosporine-methylamine-serine, mycosporine-taurine, palythene, palythine-serine, palythine-serine-sulfate, palythanol, usujirene, or a combination thereof.

In the present disclosure, the mycosporine-like amino acids may be interchangeably used with MAA and MAAs.

As used herein, the term “microorganism for producing a mycosporine-like amino acid” refers to a microorganism including a gene of an enzyme involved in the biosynthesis of mycosporine-like amino acids, or a cluster of the genes. Additionally, as used herein, the term “mycosporine-like amino acid biosynthetic gene” refers to a gene encoding an enzyme involved in the biosynthesis of mycosporine-like amino acids, and also includes a cluster of the genes. The mycosporine-like amino acid biosynthetic gene includes both foreign and/or endogenous genes of a microorganism as long as the microorganism including the gene can produce mycosporine-like amino acids. The foreign genes may be homologous and/or heterologous.

The mycosporine-like amino acid biosynthetic gene may not be limited by the species of the microorganism derived from the genes, as long as the microorganism including the same produces an enzyme involved in the biosynthesis of mycosporine-like amino acids and consequently produces mycosporine-like amino acids. Specifically, it may be *Anabaena variabilis*, *Nostoc punctiforme*, *Nodularia spumigena*, *Cyanothece* sp. PCC 7424, *Lyngbya* sp. PCC 8106, *Microcystis aeruginosa*, *Microcoleus chthonoplastes*, *Cyanothece* sp. ATCC 51142, *Crocospaera watsonii*, *Cyanothece* sp. CCY 0110, *Cylindrospermum stagnale* sp. PCC 7417, *Aphanothece halophytica* or *Trichodesmium erythraeum*, which are species of cyanobacteria, or *Magnaporthe oryzae*, *Pyrenophora tritici-repentis*, *Aspergillus clavatus*, *Nectria haematococca*, *Aspergillus nidulans*, *Gibberella zeae*, *Verticillium albo-atrum*, *Botryotinia fuckeliana*, or *Phaeosphaeria nodorum*, which are species of fungi, or *Nematostella vectensis*, *Heterocapsa triquetra*, *Oxyrrhis marina*, *Karlodinium micrum*, *Actinosynnema mirum*, etc., but is not limited thereto.

According to one embodiment, the microorganism for producing a mycosporine-like amino acid of the present disclosure may include a mycosporine-like amino acid biosynthetic gene.

Specifically, the mycosporine-like amino acid biosynthetic gene may not be limited by the name of the enzymes or the derived microorganisms as long as the microorganism can produce mycosporine-like amino acids, and may specifically include at least one, specifically one or more, two or more, or three or more, or all enzymatic proteins selected from the group consisting of 2-demethyl 4-deoxygadusol synthase, O-methyltransferase, and C-N ligase; or a gene encoding an enzymatic protein having an activity identical and/or similar thereto.

For example, the 2-demethyl 4-deoxygadusol synthase is an enzyme that converts sedoheptulose-7-phosphate into 2-demethyl-4-deoxygadusol. The O-methyltransferase is an enzyme that converts 2-demethyl-4-deoxygadusol into 4-deoxygadusol, and glycylation of the 4-deoxygadusol is catalyzed by the C-N ligase.

Additionally, the microorganism for producing a mycosporine-like amino acid may include a gene of an enzyme having an activity of attaching additional amino acid residues to mycosporine-like amino acids, or a cluster of the genes. The gene or the cluster of the genes may not be limited by the name of the enzymes or the derived microorganisms as long as the microorganism for producing a mycosporine-like amino acid can produce mycosporine-like amino acids, to which two or more amino acid residues are attached, and may specifically

include at least one, specifically one or more, two or more, three or more, or all enzymatic proteins selected from the group consisting of non-ribosomal peptide synthetase (NRPS), non-ribosomal peptide synthetase-like enzyme (NRPS-like enzyme), and D-Ala D-Ala ligase (DDL); or a gene encoding an enzymatic protein having an activity identical and/or similar thereto. Some mycosporine-like amino acids may include a second amino acid residue in mycosporine-glycine. The at least one enzyme selected from the group consisting of non-ribosomal peptide synthetase, non-ribosomal peptide synthetase-like enzyme, and D-Ala D-Ala ligase may attach a second amino acid residue to mycosporine-glycine.

According to one embodiment, the microorganism for producing a mycosporine-like amino acid may include any enzyme without limitation to the name of the enzymes or species of the derived microorganisms as long as it has an activity of attaching a second amino acid to mycosporine-glycine, as the non-ribosomal peptide synthetase, non-ribosomal peptide synthetase-like enzyme, and D-Ala D-Ala ligase.

In an embodiment, the non-ribosomal peptide synthetase-like enzyme (Ava_3855) found in *Anabaena variabilis* or D-Ala D-Ala ligase (NpF5597) found in *Nostoc punctiforme* can produce shinorine by attachment of serine residue to mycosporine-glycine. In another embodiment, mycosporine-2-glycine may be formed by attachment of a second glycine residue via a D-Ala D-Ala ligase homolog (Ap_3855) found in *Aphanothece halophytica*. Similarly, in *Actinosynnema mirum*, serine or alanine may be attached by a D-Ala D-Ala ligase to form shinorine or mycosporine-glycine-alanine. The microorganism according to one embodiment of the present disclosure may include an enzyme suitable for the production of desired mycosporine-like amino acids by selecting from the above-described enzymes or enzymes having an activity identical and/or similar thereto.

The 2-demethyl 4-deoxygadusol synthase, O-methyltransferase, C-N ligase, non-ribosomal peptide synthetase, non-ribosomal peptide synthetase-like enzyme, and/or D-Ala D-Ala ligase that can be used in the present disclosure may not be limited by the species of the derived microorganisms, and any enzyme may be included without limitation as long as it is known to serve identical and/or similar functions and activities. Additionally, the numerical range of homology between these enzymes may not be limited. For example, MylA, MylB, MylD, MylE and MylC of *Cylindrospermum stagnale* PCC 7417 are homologous to 2-demethyl 4-deoxygadusol synthase, O-methyltransferase, C-N ligase, and D-Ala D-Ala ligase derived from *Anabaena variabilis* and *Nostoc punctiforme*, and the similarity between these homologs is

about 61 to 88% (*Appl Environ Microbiol*, 2016, 82(20), 6167-6173; *J Bacteriol*, 2011, 193(21), 5923-5928). That is, the enzyme that can be used in the present disclosure may not be particularly limited by the species of the derived microorganisms or sequence homology as long as it is known to exhibit identical and/or similar functions and effects. Further, the non-patent literatures disclosed in the prior art are incorporated herein by reference in their entirety.

Additionally, the mycosporine-like amino acid biosynthetic gene may be a polynucleotide encoding a protein including the amino acid sequence of SEQ ID NO: 2, 4, 86, 88, 90, 92, 94, 96, 98, 100, 102, or 104, but is not limited thereto.

Additionally, the mycosporine-like amino acid biosynthetic gene may include a nucleotide sequence encoding a protein including the amino acid sequence having a homology of 50%, 60%, or 70% or more, specifically, 80% or more, more specifically 90% or more, even more specifically 95%, and even more specifically 99% to the amino acid sequence of SEQ ID NO: 2, 4, 86, 88, 90, 92, 94, 96, 98, 100, 102, or 104, and any nucleotide sequence encoding a protein having a homology deviating from the above may be included without limitation as long as the microorganism produce mycosporine-like amino acids. Specifically, the mycosporine-like amino acid biosynthetic gene may include, but is not limited to, the nucleotide sequence of SEQ ID NO: 1, 3, 85, 84, 89, 91, 93, 95, 97, 99, 101, or 103.

Additionally, it is apparent that, as an amino acid sequence having a homology to the above amino acid sequence, an amino acid sequence with deletion, modification, substitution, or addition of a part of the sequence also falls within the scope of the present disclosure as long as the amino acid sequence has a biological activity substantially identical or corresponding to that of the protein having the amino acid sequence of SEQ ID NO: described above.

Additionally, considering the codons preferred by organisms to express the protein due to codon degeneracy, various modifications may be executed on the coding region within the scope without changing the amino acid sequence of the polypeptide. Accordingly, the mycosporine-like amino acid biosynthetic gene may include without limitation any nucleotide sequence encoding a protein involved in the synthesis of mycosporine-like amino acids.

Additionally, a probe which can be prepared from a known gene sequence, for example, any sequence which hybridizes with a sequence complementary to all or a part of the nucleotide sequence under stringent conditions to encode a protein involved in the biosynthesis of mycosporine-like amino acids may be included without limitation.

According to an embodiment, the microorganism for producing a mycosporine-like amino acid may include mycosporine-like amino acid biosynthetic genes derived from different origins.

In the present disclosure, the inactivation of a protein, enhancement of the activity of a protein, and/or introduction of a gene herein may be performed simultaneously, sequentially, or in a reverse order.

As used herein, the term “microorganism for producing a mycosporine-like amino acid” possess endogenous and/or exogenously introduced mycosporine-like amino acid biosynthetic genes, and thus can produce mycosporine-like amino acids, and additionally, it may be a microorganism in which the ability to produce mycosporine-like amino acids is increased by inactivating the endogenous 3-dehydroquinate dehydratase activity. Introduction of the mycosporine-like amino acid biosynthetic gene and inactivation of 3-dehydroquinate dehydratase may be performed simultaneously, sequentially, or in a reverse order.

Additionally, the microorganism of the present disclosure may be a natural microorganism originally having a mycosporine-like amino acid biosynthetic gene; and a microorganism into which a heterologous and/or homologous mycosporins-like amino acid biosynthetic gene has been introduced, but is not limited thereto.

Additionally, the microorganism of the present disclosure may be a microorganism having an enhanced activity of an enzyme encoded by an endogenous and/or introduced mycosporine-like amino acid biosynthesis related gene, but is not limited thereto.

Additionally, the microorganism of the present disclosure may have no limitation as long as it has the activity of 3-dehydroquinate dehydratase before modification, and specifically, it may be a microorganism of the genus *Corynebacterium*, a microorganism of the genus *Escherichia*, or a yeast.

The microorganism of the genus *Corynebacterium* may specifically be *Corynebacterium glutamicum*, *Corynebacterium ammoniagenes*, *Brevibacterium lactofermentum*, *Brevibacterium flavum*, *Corynebacterium thermoaminogenes*, *Corynebacterium efficiens*, etc., and more specifically, it may be *Corynebacterium glutamicum*, but is not limited thereto.

The microorganism of the genus *Escherichia* may specifically be *Escherichia albertii*,

Escherichia coli, *Escherichia fergusonii*, *Escherichia hermannii*, *Escherichia vulneris*, etc., and more specifically, it may be *Escherichia coli*, but is not limited thereto.

The yeast may specifically be a microorganism belonging to Saccharomycotina, Taphrinomycotina of Ascomycota, or Agaricomycotina, Pucciniomycotina of Basidiomycota, etc., and more specifically, it may be a microorganism of the genus *Saccharomyces*, a microorganism of the genus *Schizosaccharomyces*, a microorganism of the genus of *Phaffia*, a microorganism of the genus of *Kluyveromyces*, a microorganism of the genus of *Pichia*, or a microorganism of the genus of *Candida*, and even more specifically, it may be *Saccharomyces cerevisiae*, but is not limited thereto.

In the present disclosure, the yeast for producing a mycosporine-like amino acid may be introduced with a gene encoding the 3-dehydroquinate synthase or may have an enhanced 3-dehydroquinate synthase activity. For example, if a part or all of *ARO1* is deleted in order to inactivate the 3-dehydroquinate dehydratase activity in the yeast, the function of 3-dehydroquinate synthase is lost, and thus it may be difficult to synthesize 3-DHQ. Therefore, when a part or all of the *ARO1* gene in the yeast is deleted, a gene encoding 3-dehydroquinate synthase (e.g., *aroB* gene) may be introduced, but is not limited thereto.

Another aspect of the present disclosure provides a method for producing a mycosporine-like amino acid, including:

culturing the microorganism of the present disclosure; and

recovering a mycosporine-like amino acid from the cultured microorganism or medium.

The “microorganism” and “mycosporine-like amino acids” are as described above.

As used herein, the term “culture” refers to growing the microorganism in an appropriately adjusted environment. The culture process of the present disclosure may be achieved according to an appropriate medium and culture conditions known in the art. The culture process may be easily adjusted for use by those skilled in the art according to the microorganism to be selected. The step of culturing the microorganism may be performed by a known batch culture method, continuous culture method, fed-batch culture method, etc., but is not particularly limited thereto. The medium used for culturing the microorganism of the present disclosure and other culture conditions are not particularly limited, but any medium used for the conventional culturing of the microorganism may be used. Specifically, the

microorganism of the present disclosure may be cultured in a conventional medium containing suitable carbon sources, nitrogen sources, phosphorous sources, inorganic compounds, amino acids and/or vitamins, *etc.*, in an aerobic condition while adjusting temperature, pH, *etc.* Specifically, the pH of the culture may be adjusted to a suitable pH (*e.g.*, pH 5 to 9, specifically pH 6 to 8, and most specifically pH 6.8) using a basic compound (*e.g.*, sodium hydroxide, potassium hydroxide, or ammonia) or acidic compound (*e.g.*, phosphoric acid or sulfuric acid), but is not limited thereto. Additionally, oxygen or oxygen-containing gas may be injected into the culture in order to maintain an aerobic state of the culture; or nitrogen gas, hydrogen gas or carbon dioxide gas, or no gas may be injected to maintain an anaerobic or microaerobic state, but is not limited thereto. Additionally, the culture temperature may be maintained at 20 to 45°C, specifically at 25 to 40°C, and the culturing may be performed for about 10 to 160 hours, but the culture is not limited thereto. Additionally, foam formation may be prevented during culturing using an antifoaming agent such as fatty acid polyglycol ester, *etc.*, but is not limited thereto.

Additionally, as a carbon source for the culture medium to be used, sugars and carbohydrates (*e.g.*, glucose, sucrose, lactose, fructose, maltose, molasses, starch, and cellulose), oils and fats (*e.g.*, soybean oil, sunflower seed oil, peanut oil, and coconut oil), fatty acids (*e.g.*, palmitic acid, stearic acid, and linoleic acid), alcohols (*e.g.*, glycerol and ethanol), organic acids (*e.g.*, acetic acid), *etc.* may be used alone or in combination, but is not limited thereto. As a nitrogen source, nitrogen-containing organic compounds (*e.g.*, peptone, yeast extract, meat gravy, malt extract, corn steep liquor, soybean flour, and urea) or inorganic compounds (*e.g.*, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate), *etc.* may be used alone or in combination, but is not limited thereto. As a phosphorus source, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, corresponding sodium-containing salts thereof, *etc.* may be used alone or in combination, but is not limited thereto. Further, essential growth-promoting materials such as other metal salts (*e.g.*, magnesium sulfate or iron sulfate), amino acids, vitamins, *etc.* may be contained in the medium.

The MAAs produced by the culture may be secreted into the medium or may remain in the cells.

As used herein, the term “medium” refers to a product obtained after culturing the microorganism of the present disclosure. The medium is a concept that includes both a form containing the microorganism and a form in which the microorganism is removed by

centrifugation, filtration, *etc.*, from a culture solution containing the microorganism.

In the step for recovering MAAs produced in the culturing step of the present disclosure, the desired MAAs may be collected from the culture solution using an appropriate method known in the art. For example, centrifugation, filtration, anion-exchange chromatography, crystallization, HPLC, *etc.* may be used, and the desired MAAs may be recovered from the cultured microorganism or the medium using an appropriate method known in the art. Additionally, the step for recovering MAAs may further include a separation process and/or a purification step.

[Mode for Carrying Out the Invention]

The present disclosure will be described in more detail by way of Examples. However, these Examples are given for illustrative purposes only, and the scope of the present disclosure is not intended to be limited to or by these Examples

<Construction of MAAs-Producing Recombinant Microorganisms Derived from *E. coli* and Production of MAAs Using the Same >

Example 1: Construction of Vectors Overexpressing Shinorine Biosynthetic gene Derived from Microalgae

A *A. variabilis*-based shinorine biosynthetic gene cluster is composed of four genes, that is, 2-demetyl 4-deoxygadusol synthase, O-methyltransferase, C-N ligase, and non-ribosomal peptide synthetase, and *Nostoc punctiforme*, a species of cyanobacterium, can also produce shinorine using the genes. The shinorine biosynthetic gene clusters were identified using the genomic DNA of *A. variabilis* ATCC29413 and *N. punctiforme* ATCC29133. Four vectors, each of which contains the shinorine biosynthetic genes (Ava_ABCD and Npr_ABCD) derived from *A. variabilis* ATCC29413 and *N. punctiforme* ATCC29133, respectively, were constructed using the two types of vectors pECCG 117_Ptrc_GFP_terminator and pECCG 117_Pcj1_GFP_terminator. The name of the four shinorine biosynthetic gene expression vectors, and the templates and primers used for constructing the vectors are summarized in Table 1 below.

[Table 1]

Name of Vectors	Templates used	Primers used
pECCG117_Ptrc_Ava_ABCD	<i>A. variabilis</i> ATCC29413 genomic DNA	SEQ ID NO: 5 (Forward)
pECCG117_Pcj1_Ava_ABCD		SEQ ID NO: 6 (Reverse)
pECCG117_Ptrc_Npr_ABCD	<i>N. punctiforme</i> ATCC29133 genomic DNA	SEQ ID NO: 7 (Forward)
pECCG117_Pcj1_Npr_ABCD		SEQ ID NO: 8 (Reverse)

After obtaining gene fragments using the templates and the primers, each gene fragment was ligated to the pECCG 117_Ptrc_GFP_terminator and pECCG 117_Pcj1_GFP_terminator vectors treated with EcoRV/XbaI restriction enzyme using the In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.). The expression vectors were named pECCG117_Ptrc_Ava_ABCD, pECCG117_Pcj1_Ava_ABCD, pECCG117_Ptrc_Npr_ABCD, and pECCG117_Pcj1_Npr_ABCD, respectively, and each of the expression vectors were confirmed by sequencing. The nucleotide sequences and amino acid sequences of Ava_ABCD and Npr_ABCD were specified as SEQ ID NO: 1, SEQ ID NO: 2, SE ID NO: 3, and SEQ ID NO: 4, respectively.

Example 2: Evaluation of Shinorine-Producing Ability of Strains Introduced with Shinorine Biosynthetic gene Expression Vectors

In order to confirm the MAA-producing ability in *E. coli*, four plasmids prepared in Example 1 were introduced into the W3110 strain, a wild type *E. coli*, to prepare strains with enhanced shinorine biosynthesis. The thus-produced strains were plated on a LB solid medium containing kanamycin and then cultured overnight in a 37°C incubator. One platinum loop of each strain cultured overnight in the LB solid medium was inoculated into 25 ml of a titer medium [medium composition: 40 g/L of glucose, 0.3 g/L of KH₂PO₄, 0.6 g/L of K₂HPO₄, 15 g/L of (NH₄)₂SO₄, 1 g/L of MgSO₄·7H₂O, 2.5 g/L of NaCl, 1.2 g/L of sodium citrate, 2.5 g/L of yeast extract, 40 g/L of calcium carbonate: pH 7.0], and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 2 below.

[Table 2]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
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W3110	22.3	-
W3110/ pECCG117_Ptrc_Ava_ABCD	20.1	121
W3110/ pECCG117_PCJ1_Ava_ABCD	19.8	382
W3110/ pECCG117_Ptrc_Npr_ABCD	21.0	96
W3110/ pECCG117_PCJ1_Npr_ABCD	20.2	332

As shown in Table 2 above, when the shinorine biosynthetic genes were introduced into W3110, it was confirmed that shinorine production was possible. Additionally, it was confirmed that the production of shinorine was increased by increasing the intensity of the promoters (promoter PCJ1 introduced) through the enhancement of the biosynthetic pathway.

Example 3: Construction of Strains in which 3-Dehydroquinate Dehydratase is Inactivated

Ava-A, the first gene for the biosynthesis of MAAs in microalgae, shares and uses, as substrates, DHQ (3-dehydroquinate) in the shikimate pathway and SH-7P (sedoheptulose 7-phosphate) in the pentose phosphate pathway. In order to prepare strains in which 3-dehydroquinate dehydratase is inactivated by deletion of *aroD* gene, a homologous recombination method using the lambda red recombinase was employed. As a gene insertion marker, a chloramphenicol-resistance gene of pKD3 was used, and an *aroD*-deleted cassette including a part of the *aroD* gene and the chloramphenicol-resistance gene of pKD3 plasmid was constructed by PCR using primers of SEQ ID NOS: 9 (forward) and 10 (reverse). After preparing strains (wild-type *E. coli* W3110) in which the *aroD* gene is to be deleted (SEQ ID NOS: 71 and 72), pKD46 plasmid containing a lambda red recombinase gene was transformed into the strains, and subsequently, the expression of the gene was induced using arabinose so as to prepare competent cells. The *aroD*-deleted cassette was introduced into the competent cells by electroporation, and then the cells were plated on a LB solid medium containing 30 mg/L of chloramphenicol. Thus-obtained strains were subjected to PCR using primers of SEQ ID NOS: 11 (forward) and 12 (reverse), and the *aroD* gene deletion was confirmed by observing the 1300 bp amplified-fragments.

Example 4: Evaluation of Shinorine-Producing Ability of Strains in which 3-Dehydroquinase Dehydratase is Inactivated

Two plasmids, whose expression is controlled by the PCJ1 promoter among the four plasmids prepared in Example 1, were introduced into the strains, in which the *aroD* gene is deleted, prepared in Example 3 (W3110 Δ *aroD*/pECCG117_PCJ1_Ava_ABCD and W3110 Δ *aroD* /pECCG117_PCJ1_Npr_ABCD), and then the strains were plated on a LB solid medium containing kanamycin. Subsequently, the *aroD*-deleted strains and the strains without *aroD* deletion were respectively cultured overnight in a 37°C incubator, and one platinum loop of each strain was inoculated into 25 mL of a titer medium [medium composition: 40 g/L of glucose, 0.3 g/L of KH₂PO₄, 0.6 g/L of K₂HPO₄, 15 g/L of (NH₄)₂SO₄, 1 g/L of MgSO₄·7H₂O, 2.5 g/L of NaCl, 1.2 g/L of Sodium citrate, 2.5 g/L of yeast extract, 40 g/L of calcium carbonate: pH 7.0], and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 3 below.

[Table 3]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
W3110/ pECCG117_PCJ1_Ava_ABCD	20.3	352
W3110 Δ <i>aroD</i> / pECCG117_PCJ1_Ava_ABCD	18.7	683
W3110/ pECCG117_PCJ1_Npr_ABCD	18.9	331
W3110 Δ <i>aroD</i> / pECCG117_PCJ1_Npr_ABCD	17.9	601

As shown in Table 3, the concentration of shinorine produced in the *aroD*-deleted strains was increased by 194% and 182% compared to the concentration of shinorine produced in the strains without *aroD* deletion, respectively. Accordingly, the W3110 Δ *aroD*/pECCG117_PCJ1_Ava_ABCD strain and W3110 Δ *aroD*/pECCG117_PCJ1_Npr_ABCD strain, which are *aroD*-deleted strains, were named CB06-0017 and CB06-0018 and deposited at the Korean Culture Center of Microorganisms (KCCM) under Budapest Treaty on June 26, 2017, with Accession Nos. KCCM12044P and KCCM12045P, respectively.

Example 5: Construction of Strains Having Enhanced Activity of

2-Dehydro-3-Deoxyphosphoheptonate Aldolase/Phosphoenolpyruvate

Synthetase/Transketolase I/II

In order to increase the MAA-producing ability of the MAA-producing microorganisms, the activity of 2-dehydro-3-deoxyphosphoheptonate aldolase/phosphoenolpyruvate synthetase/transketolase I/II was enhanced. Specifically, three genes derived from *E. coli* W3110, namely, *aroG* (2-dehydro-3-deoxyphosphoheptonate aldolase; SEQ ID NOS: 73 and 74), *ppsA* (phosphoenolpyruvate synthetase; SEQ ID NOS: 75 and 76), and *tktA* (transketolase I/II; SEQ ID NOS: 77 and 78) were further introduced. pSKH130- Δ fhuA-Pn-*aroG*-Pn-*ppsA*-Pn-*tktA* plasmids were constructed to enhance the *aroG*, *ppsA*, and *tktA* genes. The template and primers used in the construction of the pSKH130- Δ fhuA-Pn-*aroG*-Pn-*ppsA*-Pn-*tktA* plasmids were shown in Table 4 below.

[Table 4]

PCR Products	Template used	Primers used (Forward, Reverse)
Pn- <i>aroG</i>	W3110 genomic DNA	SEQ ID NO: 13, SEQ ID NO: 14
Pn- <i>ppsA</i>		SEQ ID NO: 15, SEQ ID NO: 16
Pn- <i>tktA</i>		SEQ ID NO: 17, SEQ ID NO: 18

Using the template and primers above, *aroG*, *ppsA*, and *tktA* gene fragments were amplified by PCR, and then respectively introduced into the pSKH130- Δ fhuA vector cleaved with the BamH1-Pst1 restriction enzyme. Sequencing was applied to confirm the cloning and the gene sequences of the vectors, and then the vectors were transformed into the wild-type *E. coli* W3110 and *aroD*-deleted *E. coli* W3110 Δ *aroD* by electroporation. The transformed genes were introduced into the chromosome by a primary recombination (crossover), followed by excision of the plasmid region from the chromosome by a secondary recombination (crossover). The introduction of *aroG*, *ppsA*, and *tktA* genes was confirmed in the *E. coli* transformed strains, in which the second recombination was completed, using primers of SEQ ID NOS: 19 (forward) and 20 (reverse).

Example 6: Evaluation of Shinorine-Producing Ability of Strains Having Enhanced Activity of 2-Dehydro-3-Deoxyphosphoheptonate Aldolase/Phosphoenolpyruvate Synthetase/Transketolase I/II

Two plasmids, whose expression is controlled by the PCJ1 promoter among the four

plasmids prepared in Example 5, were respectively introduced into the strains introduced with the *aroG*, *ppsA*, and *tktA* genes prepared in Example 5, and then the strains were plated on a LB solid medium. Subsequently, the strains were cultured overnight in a 37°C incubator, and one platinum loop of each strain was inoculated into 25 mL of the titer medium of Example 4, and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 5 below.

[Table 5]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
W3110 / pECCG117_PCJ1_Ava_ABCD	19.8	352
W3110 / pECCG117_PCJ1_Npr_ABCD	19.6	344
W3110 Δ <i>aroD</i> / pECCG117_PCJ1_Ava_ABCD	17.3	688
W3110 Δ <i>aroD</i> / pECCG117_PCJ1_Npr_ABCD	17.8	652
W3110 Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -pn- <i>tktA</i> / pECCG117_PCJ1_Ava_ABCD	18.9	1163
W3110 Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -pn- <i>tktA</i> / pECCG117_PCJ1_Npr_ABCD	18.6	989
W3110 Δ <i>aroD</i> Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -pn- <i>tktA</i> / pECCG117_PCJ1_Ava_ABCD	17.3	1928
W3110 Δ <i>aroD</i> Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -pn- <i>tktA</i> / pECCG117_PCJ1_Npr_ABCD	17.7	1889

As shown in Table 5, the concentration of shinorine produced in the strains, in which three genes (*aroG*, *ppsA*, and *tktA*) were enhanced, was increased by about 300% compared to the control group.

Example 7: Construction of *ava* ABCD Chromosomal Insertion Vectors and Strains

In order to introduce the shinorine biosynthetic genes into *E. coli*, pSKH130 Δ *pinR*::Ava-ABCD plasmids were prepared. Ava_ABCD was subjected to PCR

using a primer pair of SEQ ID NOS: 21 (forward) and 22 (reverse) based on pECCG117_Ptrc_Ava_ABCD as a template. About 7 kb PCR fragments were ligated to pSKH130 Δ pinR vector treated with BamHI and PstI restriction enzymes so as to prepare pSKH130 Δ pinR::Ava_ABCD using the In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.). Subsequently, Ptrc and PCJ1 promoter fragments were subjected to PCR using forward and reverse primer pairs of SEQ ID NOS: 23 and 24, SEQ ID NOS: 25 and 26, and SEQ ID NOS: 25 and 27, respectively, to control the expression of Ava-ABCD. Then, the fragments were ligated to pSKH130 Δ pinR::Ava_ABCD vector treated with the ScaI restriction enzyme so as to prepare pSKH130 Δ pinR::Ptrc-Ava-ABCD and pSKH130 Δ pinR::PCJ1-Ava-ABCD using the In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.). The recombinant plasmids were transformed into the W3110 Δ aroD Δ fhuA::Pn-aroG-Pn-ppsA-Pn-tktA strains prepared in Example 5 by electroporation, and the strains were introduced into the chromosome by a primary recombination (crossover), followed by excision of the vector region except the target gene from the chromosome by a secondary recombination (crossover).

The introduction of the Ava_ABCD gene was confirmed in the *E. coli* transformed strains, in which the second recombination was completed, by PCR using primers of SEQ ID NOS: 28 (forward) and 29 (reverse).

Example 8: Evaluation of Shinorine-Producing Ability of ava ABCD Chromosomal Insertion Strains

The strains prepared in Example 7 were plated on a LB solid medium and then cultured overnight in a 37°C incubator. Subsequently, one platinum loop of each strain was inoculated into 25 ml of the titer medium of Example 4, and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 6 below.

[Table 6]

Name of Strains	OD (600nm)	Shinorine Concentration (mg/L)
W3110 Δ aroD Δ fhuA::Pn-aroG-Pn-ppsA-Pn-tktA	18.5	-
W3110 Δ aroD Δ fhuA::Pn-aroG-Pn-ppsA-Pn-tktA/pECCG117_PCJ1_Ava_ABCD	17.8	1928

W3110 Δ <i>aroD</i> Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -Pn- <i>tktA</i> Δ <i>pinR</i> ::Ptrc-Ava-ABCD	18.2	483
W3110 Δ <i>aroD</i> Δ <i>fhuA</i> ::Pn- <i>aroG</i> -Pn- <i>ppsA</i> -Pn- <i>tktA</i> Δ <i>pinR</i> ::PCJ1-Ava-ABCD	17.9	832

As shown in Table 6, when Ava-ABCD was introduced into the chromosome, it was confirmed that shinorine was produced, and the concentration thereof was increased according to the intensity of the promoters. However, it was also confirmed that shinorine production was reduced compared to the strains having enhanced shinorine biosynthesis through the plasmids. When the pECCG117_PCJ1_Ava_ABCD plasmid was additionally introduced into the strains introduced with Ava-ABCD on the chromosome, shinorine production was increased by 353% and 152% compared to the strain only introduced with Ava-ABCD on the chromosome (based on the CJ1 promoter) and the strain only introduced with the plasmid, respectively.

Example 9: Construction of MAA Gene Overexpression Vectors and Evaluation of MAA-Producing Ability Thereof

4-deoxygadusol and mycosporine-glycine are intermediates that are produced during the shinorine biosynthesis and are mycosporine-like amino acids having an effect of preventing UV rays at the same time. Vectors were constructed in order to confirm whether these substances could be produced in *AroD*-deleted strains of *E. coli*. The results are shown in Table 7 below.

Ptrc_Ava_AB and Ptrc_Ava_ABC were subjected to PCR using primer pairs of SEQ ID NOS: 30 and 31 and SEQ ID NOS: 30 and 32 based on pECCG117_Ptrc_Ava_ABCD as a template. pECCG117_Ptrc_Ava_AB and pECCG117_Ptrc_Ava_ABC were prepared by ligating PCR fragments to pECCG117 Prc GFP vector treated with BamHI and SpeI restriction enzymes. In the same manner, pECCG117_PCJ1_Ava_AB and pECCG117_PCJ1_Ava_ABC were prepared by ligating the PCR fragments obtained using primers pairs of SEQ ID NOS: 30 and 31 and SEQ ID NOS: 30 and 32 based on pECCG117_PCJ1_Ava_ABCD as a template to the pECCG117 Pcj1 GFP vector treated with BamHI and SpeI restriction enzymes. The nucleotide sequence and amino acid sequence of Ava_AB and Ava_ABC are specified as SEQ ID NOS: 85 to 88.

[Table 7]

Name of Vectors	Templates used	Primers used
pECCG117_Ptrc_Ava_AB	pECCG117_Ptrc_Ava_ABCD	SEQ ID NO: 30 (Forward)
pECCG117_PCJ1_Ava_AB		SEQ ID NO: 31 (Reverse)
pECCG117_Ptrc_Ava_ABC	pECCG117_PCJ1_Ava_ABCD	SEQ ID NO: 30 (Forward)
pECCG117_PCJ1_Ava_ABC		SEQ ID NO: 32 (Reverse)

The thus-prepared vectors were transformed into W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$ strains used in Example 8 by commonly used electric-pulse method, and each strain was plated on a LB solid medium and cultured overnight in a 37 °C incubator. The strains cultured overnight in the LB solid medium were inoculated into 25 mL of the titer medium of Example 4, and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. After completion of the culture, the production of MAAs was measured by liquid high-speed chromatography, and the concentration of MAAs in the culture for each strain tested was shown in Table 8 below.

[Table 8]

Name of Strains	OD (600 nm)	4-Deoxygadusol Concentration (mg/L)	Mycosporine glycine Concentration (mg/L)
W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$	19.2	-	-
W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$ / pECCG117_Ptrc_Ava_AB	18.2	12.0	-
W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$ / pECCG117_Pcj1_Ava_AB	17.6	25.3	-
W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$ / pECCG117_Ptrc_Ava_ABC	18.7	2.0	9.3
W3110 $\triangle aroD \triangle fhuA::Pn-aroG-Pn-ppsA-Pn-tktA$ / pECCG117_Pcj1_Ava_ABC	18.1	2.9	19.7

As shown in Table 8, when the Ava_AB and Ava_ABC genes were introduced, it was confirmed that 4-deoxygadusol and mycosporine glycine were produced, and the amount thereof was increased as the intensity of the promoters was enhanced.

<Construction of MAA-Producing Recombinant Microorganisms Derived from *Corynebacterium glutamicum* and Production of MAAs Using the Same>

Example 10: Evaluation of Shinorine Producing-Ability of Strains Introduced with Shinorine Biosynthetic gene Overexpression Vectors

In order to confirm the MAA-producing ability of *Corynebacterium glutamicum*, four plasmids prepared in Example 1 were introduced into *Corynebacterium glutamicum* 13032 strain to prepare strains having enhanced shinorine biosynthesis, and the strains were plated on a BHIS solid medium containing kanamycin and cultured overnight in a 30 incubator. One platinum loop of each strain cultured overnight in the BHIS solid medium was inoculated into 25 mL of a titer medium [medium composition: 40 g/L of glucose, 1 g/L of KH_2PO_4 , 10 g/L of $(\text{NH}_4)_2\text{SO}_4$, 5 g/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g/L of NaCl, 5 g/L of yeast extract, 30 g/L of calcium carbonate: pH 7.0], and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 9 below.

[Table 9]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
c.gl 13032	72.1	-
c.gl 13032/ pECCG117_Ptrc_Ava_ABCD	71.5	132
c.gl 13032/ pECCG117_PCJ1_Ava_ABCD	69.8	496
c.gl 13032/ pECCG117_Ptrc_Npr_ABCD	70.9	103
c.gl 13032/ pECCG117_PCJ1_Npr_ABCD	71.4	421

As shown in Table 9, it was confirmed that shinorine production was possible when the shinoinine biosynthetic genes were introduced into *Corynebacterium glutamicum* 13032, and the production thereof could be increased by up to 375% depending on the intensity of the promoters.

Example 11: Construction of Vectors Introduced with Shinorine Biosynthetic gene on the Chromosome and Strains

In order to introduce the shinorine biosynthetic genes into *Corynebacterium glutamicum*, pDC ΔN1021_Ava_ABCD plasmids was prepared. Ava_ABCD was subjected to PCR using a primer pair of SEQ ID NOS: 33 (forward) and 34 (reverse) based on pECCG117_Ptrc_Ava_ABCD as a template. About 7 kb PCR fragments were ligated to pDC ΔN1021 vector treated with the NdeI restriction enzyme so as to prepare pDC ΔN1021_Ava_ABCD using the In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.). Subsequently, CJ7, Lysc8, and O2 promoter fragments were subjected to PCR using forward and reverse primer pairs of SEQ ID NOS: 35 and 36, SEQ ID NOS: 37 and 38, and SEQ ID NOS: 39 and 40, respectively, in order to control the expression of Ava-ABCD. Then, the fragments were ligated to pDC ΔN1021_Ava_ABCD vector treated with the NdeI restriction enzyme to prepare pDC ΔN1021_Pcj7_Ava_ABCD, pDC ΔN1021_Plysc8_Ava_ABCD and pDC ΔN1021_PO2_Ava_ABCD using the In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.).

The recombinant plasmids were transformed into *Corynebacterium glutamicum* 13032 by electroporation (van der Rest et al. 1999), and introduced into the chromosome by a primary recombination (crossover), followed by plasmid excision from the chromosome by a secondary recombination (crossover).

The introduction of the Ava_ABCD gene was confirmed in the *Corynebacterium glutamicum* transformed strains, in which the second recombination was completed, by PCR using a gene-specific primer pair of SEQ ID NOS: 33 (forward) and 34 (reverse).

Example 12: Evaluation of Shinorine-Producing Ability of Strains Introduced with Shinorine Biosynthetic gene on the Chromosome

All strains were plated on a BHIS solid medium and then cultured overnight in a 30 incubator to confirm the shinorine-producing ability. One platinum loop of each strain cultured overnight in the BHIS solid medium was inoculated into 25 mL of the titer medium of Example 11, and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 10 below.

[Table 10]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
-----------------	-------------	--------------------------------

c.gl 13032	70.2	-
c.gl 13032 Δ N1021_PCJ7_Ava_ABCD	76.1	36
c.gl 13032 Δ N1021_Plysc8_Ava_ABCD	79.8	75
c.gl 13032 Δ N1021_PO2_Ava_ABCD	72.5	173

As shown in Table 10, it was confirmed that, when a single copy of the shinorine biosynthetic genes was introduced into the wild-type *Corynebacterium glutamicum*, shinorine could be produced in an amount from 36 mg to 173 mg.

Example 13: Construction of *Corynebacterium aroD* (3-Dehydroquinatase)-Deleted Vectors and Strains

As mentioned in Example 3, strains with deletion were prepared to confirm whether shinorine production could be increased through the deletion of *aroD* (3-dehydroquinatase dehydratase). In order to prepare a site-specific *aroD* gene (SEQ ID NOS: 79 and 80)-deleted strains of *Corynebacterium glutamicum*, pDC- Δ *aroD* plasmids, in which an open reading frame of *aroD* was endogenously deleted, were constructed. The endogenous gene deletion of the pDC- Δ *aroD* was generated by introducing gene fragments generated by crossover-PCR using forward and reverse primer pairs of SEQ ID NOS: 41 and 42, and SEQ ID NOS: 43 and 44, based on the genomic DNA of *Corynebacterium glutamicum* ATCC 13032 as a template into the pDC vector. The recombinant plasmids were transformed into *Corynebacterium glutamicum* 13032 Δ N1021_PO2_Ava_ABCD by electroporation (van der Rest et al. 1999), and the plasmids were introduced into the chromosome by a primary recombination (crossover), followed by plasmid excision from the chromosome by a secondary recombination (crossover).

The deletion of *aroD* gene was confirmed in the *Corynebacterium glutamicum* transformed strains, in which the second recombination was completed, by PCR using a gene-specific primer pair of SEQ ID NOS: 41 and 44.

Example 14: Evaluation of *aroD* (3-Dehydroquinatase Dehydratase) Deletion

Strains expected to have DHQ accumulation due to deletion of 3-dehydroquinatase dehydratase in the *Corynebacterium glutamicum* 13032 Δ N1021_PO2_Ava_ABCD strain were plated on a BHIS solid medium and cultured overnight in a 30 incubator. One platinum loop of

each strain cultured overnight in the BHIS solid medium was inoculated into 25 mL of a titer medium [medium composition: 40 g/L of glucose, 1 g/L of KH₂PO₄, 10 g/L of (NH₄)₂SO₄, 5 g/L of MgSO₄·7H₂O, 5 g/L of NaCl, 5 g/L of yeast extract, 30 g/L of calcium carbonate: pH7.0], and then cultured in an incubator at 37°C at a rate of 200 rpm for 48 hours. The results are shown in Table 11 below.

[Table 11]

Name of Strains	OD (600 nm)	Shinorine Concentration (mg/L)
c.gl 13032 Δ N1021_PO2_Ava_ABCD	71.3	182
c.gl 13032 Δ N1021_PO2_Ava_ABCD_ Δ aroD	74.1	435
c.gl 13032 Δ N1021_PO2_Ava_ABCD_ Δ aroD /pECCG117_PCJ1_Ava_ABCD	73.2	1162

As shown in Table 11, it was confirmed that when the *aroD* gene was deleted, the concentration of shinorine was improved by 239% compared to the control group, and as the biosynthesis of shinorine was further enhanced through the pECCG117_PCJ1_Ava_ABCD, the concentration of shinorine was increased. Accordingly, the *aroD*-deleted strain, c.gl 13032 N1021_PO2_Ava_ABCD_ Δ aroD, was named CB06-0019 and deposited at the Korean Culture Center of Microorganisms (KCCM) under Budapest Treaty on June 26, 2017, with Accession No. KCCM12046P.

<Construction of MAA-Producing Recombinant Microorganisms Derived from Yeast and Production of MAAs Using the Same>

Example 15: Construction of Shinorine Biosynthetic gene Overexpression Yeast Vectors Derived from Microalgae

S.cerevisiae vectors, into which the shinorine biosynthetic genes were introduced based on the genomic DNA of *A. variabilis* ATCC29413 and *N. punctiforme* ATCC29133, were constructed. The vectors were prepared using the ADH, TEF, and GPD promoters of *S. cerevisiae*, and the templates and primers used to prepare a total of twenty-four shinorine biosynthetic gene expression vectors are shown in Table 12 below. Nucleotide sequences and amino acid sequences of Ava_A, Ava_B, Ava_C, Ava_D, Npr_A, Npr_B, Npr_C, and Npr_D

are specified as SEQ ID NOS: 89 to 104 in the order of the enzymes.

[Table 12]

Name of Vectors	Templates Used	Primers used (Forward, Reverse)
p413-pADH-Ava_A	<i>A. variabilis</i> ATCC29413 genomic DNA	SEQ ID NO: 45, SEQ ID NO: 46
p413-pADH-Ava_B		SEQ ID NO: 47, SEQ ID NO: 48
p413-pADH-Ava_C		SEQ ID NO: 49, SEQ ID NO: 50
p413-pADH-Ava_D		SEQ ID NO: 51, SEQ ID NO: 52
p413-pTEF-Ava_A		SEQ ID NO: 45, SEQ ID NO: 46
p413-pTEF-Ava_B		SEQ ID NO: 47, SEQ ID NO: 48
p413-pTEF-Ava_C		SEQ ID NO: 49, SEQ ID NO: 50
p413-pTEF-Ava_D		SEQ ID NO: 51, SEQ ID NO: 52
p413-pGPD-Ava_A		SEQ ID NO: 45, SEQ ID NO: 46
p413-pGPD-Ava_B		SEQ ID NO: 47, SEQ ID NO: 50
p413-pGPD-Ava_C		SEQ ID NO: 49, SEQ ID NO: 50
p413-pGPD-Ava_D		SEQ ID NO: 51, SEQ ID NO: 52
p413-pADH-Npr_A	<i>N.punctiforme</i> ATCC29133 genomic DNA	SEQ ID NO: 53, SEQ ID NO: 54
p413-pADH-Npr_B		SEQ ID NO: 55, SEQ ID NO: 56
p413-pADH-Npr_C		SEQ ID NO: 57, SEQ ID NO: 58
p413-pADH-Npr_D		SEQ ID NO: 59, SEQ ID NO: 60
p413-pTEF-Npr_A		SEQ ID NO: 53, SEQ ID NO: 54
p413-pTEF-Npr_B		SEQ ID NO: 55, SEQ ID NO: 56
p413-pTEF-Npr_C		SEQ ID NO: 57, SEQ ID NO: 58
p413-pTEF-Npr_D		SEQ ID NO: 59, SEQ ID NO: 60
p413-pGPD-Npr_A		SEQ ID NO: 53, SEQ ID NO: 54
p413-pGPD-Npr_B		SEQ ID NO: 55, SEQ ID NO: 56
p413-pGPD-Npr_C		SEQ ID NO: 57, SEQ ID NO: 58
p413-pGPD-Npr_D		SEQ ID NO: 59, SEQ ID NO: 60

The gene fragments obtained by PCR using the combination of templates and primers above were ligated to p413/414/415/416-pADH/pTEF/pGPD-CYC1_terminator vector treated with the BamH1/XhoI restriction enzyme using the T4 ligase enzyme (NEB), thereby preparing twenty-four p413/414/415/416-pADH/pTEF/pGPD-A, B, C, D vectors. Construction of each expression vector and gene sequences thereof were confirmed by sequencing techniques. The thus-prepared expression vectors were introduced into a wild-type *S. cerevisiae* CEN.PK-1D strain so as to prepare strains capable of producing shinorine.

Example 16: Evaluation of Shinorine-Producing Ability of Strains Introduced with Shinorine Biosynthetic Gene Overexpression Vectors

In order to confirm the MAA-producing ability of yeasts, twenty-four plasmids prepared in Example 15 were introduced into the *Saccharomyces cerevisiae* CEN.PK-1D strain (*S. cerevisiae* CEN.PK-1D) to prepare strains having enhanced shinorine biosynthesis, and the strains were plated on a SC (synthetic complete) solid medium excluding Leu, Trp, Ura, and His and cultured overnight in a 30 incubator. One platinum loop of each strain cultured overnight was inoculated into 25 mL of the titer medium shown in Table 13 and then cultured in an incubator at 30 at a rate of 150 rpm for 24 hours. The results are shown in Table 14 below.

[Table 13]

Composition	Concentration (g/L)
YNB(Yeast nitrogen base) without amino acids	6.7
Amino acid mixtures (without Leucine, Tryptophan, Histidine, Uracil)	2
Glucose	20

[Table 14]

Plasmid	24 hr		Shinorine Concentration (mg/L)
	OD ₆₀₀	Residual Sugar	
pADH-Ava_A,B,C,D	11.0	0.0	107

pTEF-Ava_A,B,C,D	11.1	0.0	215
pGPD-Ava_A,B,C,D	11.5	0.0	302
pADH-Npr_A,B,C,D	20.1	0.0	234
pTEF-Npr_A,B,C,D	20.4	0.0	387
pGPD-Npr_A,B,C,D	20.5	0.0	521

Based on the results above, it was confirmed that the *S. cerevisiae* CEN.PK-1D (*i.e.*, a yeast strain) showed a high activity for Npr A, B, C, D genes compared to Ava A, B, C, D genes. Additionally, it is confirmed that the expression level of the genes was controlled according to the intensity of the promoters, and thus, the production of shinorine was altered. In particular, it was confirmed that the shinorine production was the highest (521 mg/L) when the GPD (Glyceraldehyde-3-phosphate dehydrogenase) promoter-based Npr A, B, C, D vector was introduced.

Example 17: Increase of Shinorine Production by *ARO1* Deletion in *S. cerevisiae* and Introduction of *E. coli aroB*

In order to determine whether the inactivation of dehydroquinate dehydratase improves shinorine production in yeasts, the *ARO1* gene was deleted in *S. cerevisiae* CEN.PK-1D. The *ARO1* gene of *S. cerevisiae* is a gene that serves five functions, and the function of 3-dehydroquinate synthase corresponding to *E. coli aroB* is lost upon deletion of the *ARO1* gene, making the synthesis of 3-DHQ impossible. Therefore, after deletion of the *S. cerevisiae ARO1* gene (SEQ ID NOS: 81 and 82), which is an *E. coli aroB* homologue on the chromosome, the *E. coli aroB* gene (SEQ ID NOS: 83 and 84) was inserted at the same position based on the GPD promoter. The templates and primers used are shown in Table 15. The twenty-four plasmids prepared in Example 15 were introduced into the *S. cerevisiae* CEN.PK-1D strain, in which the *ARO1* gene is deleted and the *E. coli aroB* gene is introduced, and the strains were plated on a SC (synthetic complete) solid medium excluding Leu, Trp, Ura, and His and cultured overnight in a 30 incubator. One platinum loop of each strain cultured overnight was inoculated into 25 mL of the titer medium shown in Table 13 and then cultured in an incubator at 30 at a rate of 150 rpm for 24 hours. The results are shown in Table 16 below.

[Table 15]

Name of Cassette	Amplified DNA	Template	Sequence Nos. (Forward, Reverse)
<i>ARO1</i> ΔpGPD-aroB cassette	pGPD	<i>S.cerevisiae</i> gDNA	SEQ ID NO: 61, SEQ ID NO: 62
	LoxP(Ura)	pUG28 vector	SEQ ID NO: 63, SEQ ID NO: 64
	aroB	W3110 gDNA	SEQ ID NO: 65, SEQ ID NO: 66
	<i>ARO1</i> Fragment1	<i>S.cerevisiae</i> gDNA	SEQ ID NO: 67, SEQ ID NO: 68
	<i>ARO1</i> Fragment2	<i>S.cerevisiae</i> gDNA	SEQ ID NO: 69, SEQ ID NO: 70

[Table 16]

Plasmids	24 hr		Shinorine Concentration (mg/L)
	OD ₆₀₀	Residual Sugar	
WT pADH-Ava_A,B,C,D	11.0	0.0	103
WT pTEF-Ava_A,B,C,D	11.1	0.0	235
WT pGPD-Ava_A,B,C,D	11.5	0.0	323
WT pADH-Npr_A,B,C,D	20.1	0.0	230
WT pTEF-Npr_A,B,C,D	20.4	0.0	390
WT pGPD-Npr_A,B,C,D	20.5	0.0	545
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pADH-Ava_A,B,C,D	13.0	0.0	305
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pTEF-Ava_A,B,C,D	13.1	0.0	635
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pGPD-Ava_A,B,C,D	13.3	0.0	912
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pADH-Npr_A,B,C,D	24.1	0.0	670
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pTEF-Npr_A,B,C,D	25.3	0.0	1230
<i>S. cerevisiae</i> <i>ARO1</i> ΔpGPD-aroB, pGPD-Npr_A,B,C,D	24.8	0.0	1600

Based on the results shown in Table 16, it was confirmed that shinorine production was increased by 3-fold in the strains, in which the DHQ-producing ability was enhanced by *ARO1*

deletion and *E. coli aroB* introduction, compared to the WT strains. Additionally, it was confirmed that the strains showed a high activity for Npr A, B, C, D genes compared to Ava A, B, C, D genes, and that the shinorine production was increased as the intensity of the promoters increased. In particular, it was confirmed that the shinorine production was the highest (1.6 g/L) when the GPD promoter-based Npr A, B, C, D vector was introduced.

Those skilled in the art will recognize that the present disclosure may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the present disclosure is therefore indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within the scope of the present disclosure.



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To, CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400,
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

1. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : <i>Escherichia coli</i> CB05-0017	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12044P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on June. 26, 2017. (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 120-861 Republic of Korea	Signature (s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: June. 26, 2017.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : <i>Escherichia coli</i> CB06-0018	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12045P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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BUDAPEST TREATY ON THE INTERNATIONAL
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : <i>Corynebacterium glutamicum</i> CB06-0019	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12046P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on June. 26. 2017. (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 120-861 Republic of Korea	Signature (s) of person (s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: June. 26. 2017.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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한국미생물보존센터

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[CLAIMS]

[Claim 1]

A microorganism for producing a mycosporine-like amino acid, wherein an activity of 3-dehydroquinate dehydratase is inactivated as compared to a non-modified microorganism.

[Claim 2]

The microorganism of claim 1, wherein the microorganism comprises a gene encoding at least one protein selected from the group consisting of 2-demetyl 4-deoxygadusol synthase, O-methyltransferase, and C-N ligase.

[Claim 3]

The microorganism of claim 1, wherein the microorganism comprises a gene encoding at least one protein selected from the group consisting of non-ribosomal peptide synthetase, non-ribosomal peptide synthetase-like enzyme (NRPS-like enzyme), and D-Ala D-Ala ligase.

[Claim 4]

The microorganism of claim 1, wherein an activity of at least one protein selected from the group consisting of 2-dehydro-3-deoxyphosphoheptonate aldolase, phosphoenolpyruvate synthetase, transketolase I/II, and 3-dehydroquinate synthase is further enhanced as compared to a non-modified microorganism.

[Claim 5]

The microorganism of claim 1, wherein the microorganism is a microorganism of the genus *Corynebacterium*, a microorganism of the genus *Escherichia*, or a yeast.

[Claim 6]

The microorganism of claim 5, wherein the yeast is introduced with a gene encoding 3-dehydroquinate synthase.

[Claim 7]

The microorganism of claim 1, wherein the mycosporine-like amino acid is at least one selected from the group consisting of mycosporine-2-glycine, palythanol, palythenic acid,

deoxygadusol, mycosporine-methylamine-threonine, mycosporine-glycine-valine, palythine, asterina-330, shinorine, porphyra-334, euhalothece-362, mycosporine-glycine, mycosporine-ornithine, mycosporine-lysine, mycosporine-glutamic acid-glycine, mycosporine-methylamine-serine, mycosporine-aurine, palythene, palythine-serine, palythine-serine-sulfate, palythanol, and usujirene.

[Claim 8]

A method for producing a mycosporine-like amino acid, comprising:

culturing the microorganism of any one of claims 1 to 7; and

recovering a mycosporine-like amino acid from the cultured microorganism or medium.