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(54) **NOVEL PHOSPHOENOLPYRUVATE CARBOXYLASE VARIANT AND METHOD FOR PRODUCING 5'-INOSINIC ACID USING SAME**

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(57) **ABSTRACT**

The present invention relates to a novel phosphoenolpyruvate carboxylase variant and a method of producing 5'-inosinic acid using the same. The phosphoenolpyruvate carboxylase variant is obtained by substituting one or more amino acids in the amino acid sequence constituting phosphoenolpyruvate carboxylase to change the activity of the protein, and a recombinant microorganism comprising the phosphoenolpyruvate carboxylase variant is capable of efficiently producing 5'-inosinic acid.

Specification includes a Sequence Listing.

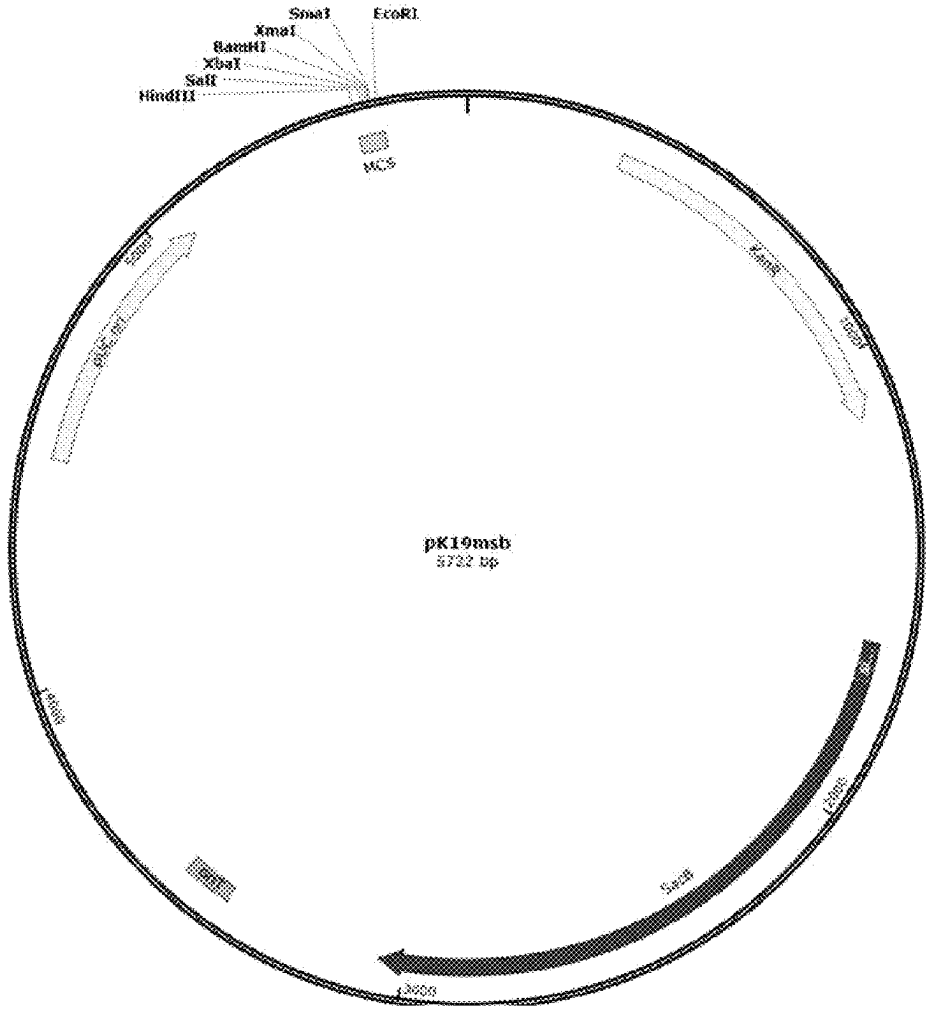
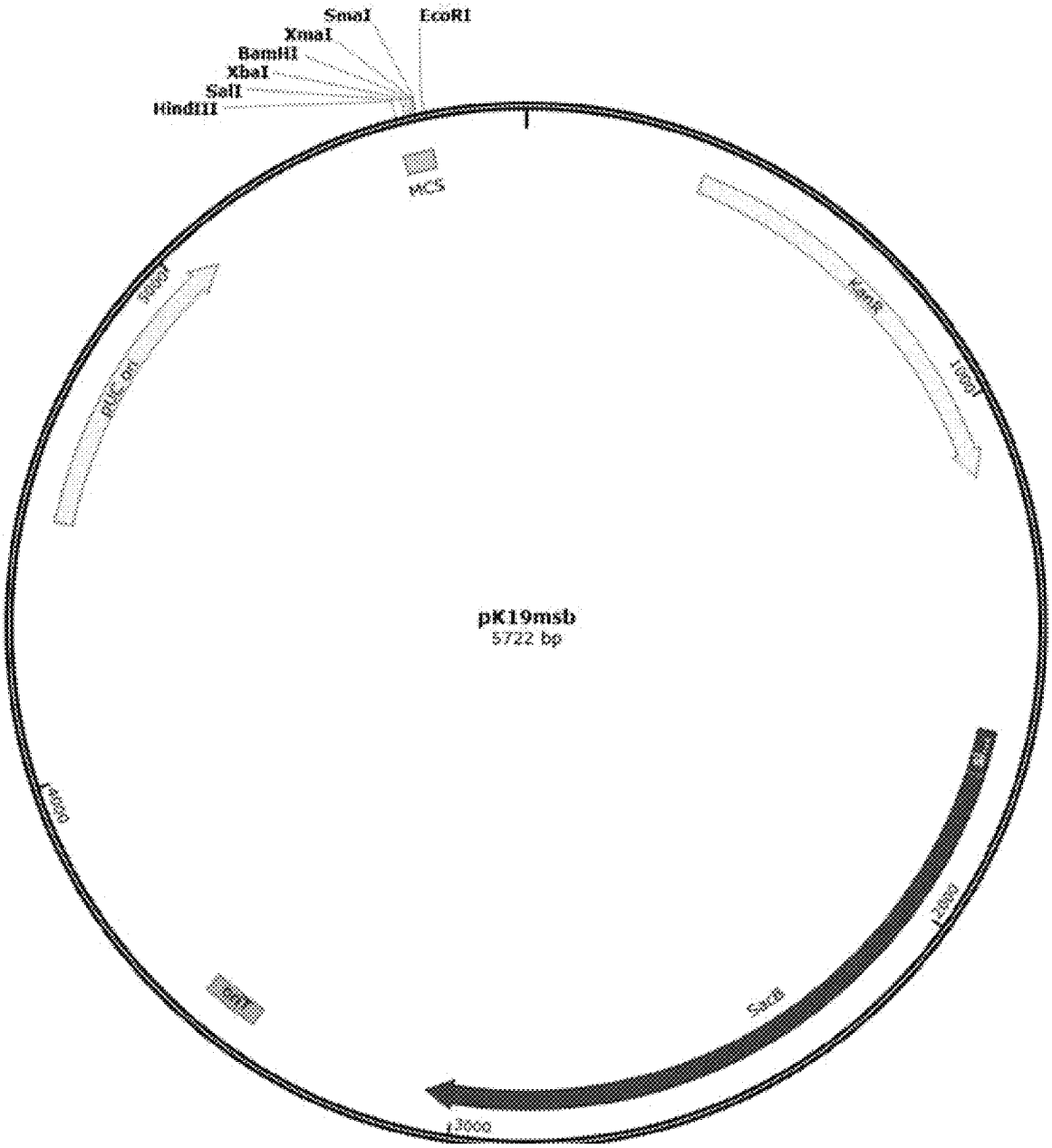


Fig.1


SmaI
EcoRI
XmaI
BamHI
XbaI
SalI
HindIII
pK19msb
5722 bp
MCS
lacZ
KanR
SacB
1000
2000
3000
4000
5000

NOVEL PHOSPHOENOLPYRUVATE CARBOXYLASE VARIANT AND METHOD FOR PRODUCING 5'-INOSINIC ACID USING SAME

TECHNICAL FIELD

[0001] The present invention relates to a novel phosphoenolpyruvate carboxylase variant and a method of producing 5'-inosinic acid using the same.

BACKGROUND ART

[0002] 5'-inosinic acid (or inosine monophosphate (IMP)) is an intermediate in the metabolic system of nucleic acid biosynthesis, and not only plays an important physiological role in the bodies of plants and animals, but is also used in various applications, including food, medicine, and various medical applications. In particular, 5'-inosinic acid is a nucleic acid-based seasoning, which has drawn much attention as a savory seasoning, because it has significant synergistic effects on taste when used together with monosodium glutamate (MSG).

[0003] Methods for producing 5'-inosinic acid include a method of enzymatically degrading ribonucleic acid extracted from yeast cells, a method of chemically phosphorylating inosine produced by fermentation, etc. Recently, a method of culturing a 5'-inosinic acid-microorganism and recovering 5'-inosinic acid producing accumulated in the medium has been mainly used.

[0004] For the production of 5'-inosinic acid using microorganisms, in order to improve the efficiency of production of 5'-inosinic acid, there has been development of a variety of recombinant strains or mutant strains having excellent 5'-inosinic acid productivity by applying genetic recombination technology to microorganisms such as *Escherichia coli* and *Corynebacterium*, which are widely used in the production of useful substances such as nucleic acids or L-amino acids, and methods of producing 5'-inosinic acid using the same. In particular, there have been attempts to increase the production of 5'-inosinic acid by targeting genes such as enzymes, transcription factors and transport proteins, which are involved in the biosynthetic pathway of 5'-inosinic acid, or by inducing mutations in promoters that regulate the expression of these genes. However, there are dozens to hundreds of types of proteins such as enzymes, transcription factors and transport proteins, which involved directly or are indirectly in the production of 5'-inosinic acid, and thus much research is still needed on the increase in 5'-inosinic acid productivity by changes in the activity of these proteins.

PRIOR ART DOCUMENTS

Patent Documents

[0005] Korean Patent No. 10-116602

DISCLOSURE

Technical Problem

[0006] An object of present invention is to provide a novel phosphoenolpyruvate carboxylase variant.

[0007] Another object of the present invention is to provide a polynucleotide encoding the variant.

[0008] Still another object of the present invention is to provide a transformant comprising the variant Or polynucleotide.

[0009] Yet another object of the present invention is to provide a method of producing 5'-inosinic acid using the transformant.

Technical Solution

[0010] One aspect of the present invention provides a phosphoenolpyruvate carboxylase variant in which one or more of the amino acids at positions 472 and 673 in the amino acid sequence of SEQ ID NO: 4 is/are substituted with other amino acid(s).

[0011] As used in the present invention, the term "phosphoenolpyruvate carboxylase" catalyzes a reaction of removing carbon dioxide from phosphoenolpyruvate, and it may be a polypeptide or protein consisting of the amino acid sequence of SEQ ID NO: 4 and having phosphoenolpyruvate carboxylase activity.

[0012] Information on the nucleic acid and protein sequences of the phosphoenolpyruvate carboxylase is available from known sequence databases (e.g., GenBank, UniProt).

[0013] According to one embodiment of the present invention, the phosphoenolpyruvate carboxylase may be encoded by the nucleotide sequence of SEQ ID NO: 3.

[0014] The amino acid sequence of the phosphoenolpyruvate carboxylase according to the present invention or the nucleotide sequence encoding the same may include a nucleotide sequence or amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% homology or identity to the amino acid sequence of SEQ ID NO: 4 or the nucleotide sequence of SEQ ID NO: 3. As used herein, the term "homology" or "identity" means the percentage rate of identity between two sequences, which is determined by aligning the reference nucleotide sequence or amino acid sequence and any other nucleotide sequence of amino acid sequence to correspond to each other as much as possible and analyzing the aligned sequences.

[0015] According to one embodiment of the present invention, the phosphoenolpyruvate carboxylase may be derived from wild-type *Corynebacterium stationis*.

[0016] As used in the present invention, the term "variant" refers to a protein that has an amino acid sequence different from the amino acid sequence before mutation by the conservative substitution and/or modification of one or more amino acids at the N-terminus, C-terminus of and/or within the amino acid sequence, which result(s) from mutation in the nucleotide sequence of the gene encoding the protein, but retains the functions or properties of the protein before mutation. As used herein, the term "conservative substitution" means substituting one amino acid with another amino acid having similar structural and/or chemical properties. The conservative substitution may have little or no impact on the activity of the protein or polypeptide. In addition, the term "modification" refers to substitution, insertion, deletion, or the like of one or more amino acids. The amino acid is selected from among alanine (Ala, A), isoleucine (Ile, I), valine (Val, V), leucine (Leu, L), methionine (Met, M), asparagine (Asn, N), cysteine (Cys, C), glutamine (Gln, Q), serine (Ser, S), threonine (Thr, T), phenylalanine (Phe, F), tryptophan (Trp, W), tyrosine (Tyr, Y), aspartic acid (Asp,

D), glutamic acid (Glu, E), arginine (Arg, R), histidine (His, H), lysine (Lys, K), glycine (Gly, G), and proline (Pro, P).

[0017] In addition, some variants include those in which one or more portions, such as an N-terminal leader sequence of transmembrane domain, have been removed, or those in which a portion has been removed from the N- and/or C-terminus of a mature protein.

[0018] The variant may have increased (enhanced), unchanged, or decreased (weakened) ability compared to that of the protein before mutation. Here, the term "increased or enhanced" includes: a case in which the activity of the protein itself has increased compared to the activity of the protein before mutation; a case in which the overall activity of the protein in the cell is higher than that in the wild-type strain or the strain expressing the protein before mutation due to increased expression or translation of the gene encoding the protein; and a combination thereof. In addition, the term "decreased or weakened" includes: a case in which the activity of the protein itself has decreased compared to the activity of the protein before mutation; a case in which the overall activity of the protein in the cell is lower than that in the wild-type strain or the strain expressing the protein before mutation due to reduced expression or translation of the gene encoding the protein; and a combination thereof. In the present invention, the term "variant" may be used interchangeably with terms such as variant type, modification, variant polypeptide, mutated protein, mutation, and the like.

[0019] According to one embodiment of the present invention, the variant may consist of the amino acid sequence of SEQ ID NO: 2 in which proline at position 472 in the amino acid sequence of SEQ ID NO: 4 is substituted with serine and glycine at position 673 is substituted with aspartic acid.

[0020] More specifically, the phosphoenolpyruvate carboxylase variant may comprise an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% homology or identity to the amino acid sequence of SEQ ID NO: 2.

[0021] Another aspect of the present invention provides a polynucleotide encoding the phosphoenolpyruvate carboxylase variant.

[0022] As used in the present invention, the term "polynucleotide" refers to a DNA or RNA strand having a certain length or more, which is a long-chain polymer of nucleotides formed by linking nucleotide monomers via covalent bonds. More specifically, the term "polynucleotide" refers to a polynucleotide fragment encoding the variant.

[0023] According to one embodiment of the present invention, the polynucleotide may comprise nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 2.

[0024] More specifically, the polynucleotide may comprise the nucleotide sequence of SEQ ID NO: 1 in which nucleotide "c" at position 1414 in the nucleotide sequence of SEQ ID NO: 3 encoding phosphoenolpyruvate carboxylase is substituted with nucleotide "t" and nucleotide "g" at position 2018 is substituted with nucleotide "a".

[0025] Still another aspect of the present invention provides a vector comprising a polynucleotide encoding the phosphoenolpyruvate carboxylase variant.

[0026] Yet another aspect of the present invention provides a transformant comprising the phosphoenolpyruvate carboxylase variant or the polynucleotide.

[0027] As used in the present invention, the term "vector" refers to any type of nucleic acid sequence transfer structure that is used as a means for transferring and expressing a gene of interest in a host cell. Unless otherwise specified, the term "vector" may mean one allowing the nucleic acid sequence contained therein to be expressed after insertion into the host cell genome and/or one allowing the nucleic acid sequence to be expressed independently. This vector comprises essential regulatory elements operably linked so that an inserted gene can be expressed. As used herein, the term "operably linked" means that a gene of interest and regulatory sequences thereof are functionally linked together in a manner enabling gene expression, and the "regulatory elements" include a promoter for initiating transcription, any operator sequence for regulating transcription, a sequence encoding suitable mRNA ribosome-binding sites, and a sequence for regulating termination of transcription and translation.

[0028] The vector in the present invention is not particularly limited as long as it may replicate in a host cell, and any vector known in the art may be used, Examples of the vector include a natural or recombinant plasmid, cosmid, virus and bacteriophage. Examples of a phage vector or cosmid vector include, but are not limited to, pWE15, M13, λMBL3, λMBL4, λIXII, λASHII, λAPII, λt10, λt11, Charon4A, and Charon21A, and examples of a plasmid vector include, but are not limited to, pBR series, pUC series, pBluescriptII series, pGEM series, pTZ series, pCL series, and pET series.

[0029] The vector may typically be constructed as a vector for cloning or as a vector for expression. The vector for expression may be a conventional vector that is used in the art to express a foreign gene or protein in a plant, animal, or microorganism, and may be constructed through various methods known in the art.

[0030] As used in the present invention, the term "recombinant vector" may be transformed into a suitable host cell, and then may replicate regardless of the genome of the host cell or may be integrated into the genome itself. In this case, the "suitable host cell" may contain a replication origin, which is a particular nucleotide sequence which enables the vector to replicate in the suitable host cell and from which replication starts. For example, when the vector used is an expression vector and uses a prokaryotic cell as a host, the vector generally comprises a strong promoter capable of promoting transcription (e.g., pLλ promoter, CMV promoter, trp promoter, lac promoter, tac promoter, T7 promoter, etc.), a ribosome binding site for initiation of translation, and a transcription/translation termination sequence. When a eukaryotic cell is used as a host, the vector comprises a replication origin operating in the eukaryotic cell, and examples of the replication origin include, but are not limited to, an f1 replication origin, an SV40 replication origin, a pMB1 replication origin, an adeno replication origin, an AAV replication origin, and a BBV replication origin. In addition, the recombinant vector may comprise a promoter derived from the genome of a mammalian cell (e.g., metallothionein promoter) or a promoter derived from a mammalian virus (e.g., adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus promoter, HSV-tk promoter, etc.), and generally has polyadenylation sequence transcription termination sequence.

[0031] The recombinant vector may comprise a selection marker. The selection marker serves to select a transformant (host cell) transformed with the vector, and since only cells expressing the selection marker can survive in the medium treated with the selection marker, it is possible to select

transformed cells. Representative examples of the selection marker include, but are not limited to, kanamycin, streptomycin, and chloramphenicol.

[0032] The transformant may be produced by inserting the recombinant vector into a host cell, and the transformant may be obtained by introducing the recombinant vector into an appropriate host cell. The host cell is a cell capable of stably and continuously cloning or expressing the expression vector, and any host cell known in the art may be used.

[0033] Where the vector is transformed into prokaryotic cells to generate recombinant microorganisms, examples of host cells that may be used include, but are not limited to, *E. coli* sp. strains such as *E. coli* DH5α, *E. coli* JM109, *E. coli* BL21, *E. coli* RR1, *E. coli* LE392, *E. coli* B, *E. coli* X 1776, *E. coli* W3110, and *E. coli* XL1-Blue, *Bacillus* sp. strains such as *Bacillus subtilis Bacillus* and *thuringiensis, Corynebacterium* sp. strains such as *Corynebacterium glutamicum* and *Corynebacterium stationis*, and various Enterobacteriaceae strains such as *Salmonella typhimurium, Serratia marcescens*, and *Pseudomonas* species.

[0034] Where the vector is transformed into eukaryotic cells to generate recombinant microorganisms, examples of host cells that may be used include, but are not limited to, yeast (e.g., *Saccharomyces cerevisiae*), insect cells, plant cells and animal cells, such as Sp2/0, CHO K1, CHO DG44, PER.C6, W138, BHK, COS7, 293, HepG2, Huh7, 3T3, RIN, and MDCK cell lines.

[0035] As used in the present invention, the term "transformation" refers to a phenomenon in which external DNA is introduced into a host cell, thereby artificially causing genetic changes, and the term "transformant" refers to a host cell into which external DNA has been introduced and in which the expression of the gene of interest is stably maintained.

[0036] The transformation may be performed using a suitable vector introduction technique selected depending on the host cell, so that the gene of interest or a recombinant vector comprising the same may be expressed in the host cell. For example, introduction of the vector may be performed by electroporation, heat-shock, calcium phosphate ($CaPO_4$) precipitation, calcium chloride ($CaCl_2$) precipitation, microinjection, polyethylene glycol (PEG) method, DEAE-dextran method, cationic liposome method, lithium acetate-DMSO method, or any combination thereof, without being limited thereto. As long as the transformed gene may be expressed in the host cell, it may be inserted into the chromosome of the host cell, or exist extrachromosomally, without being limited thereto.

[0037] The transformant may include a cell transfected, transformed, or infected with the recombinant vector of the present invention in vivo or in vitro, and may be used in the same sense as a recombinant host cell, a recombinant cell, or a recombinant microorganism.

[0038] Genes inserted into the recombinant vector of the present invention may be introduced into a host cell such as a *Corynebacterium* sp. strain by homologous recombination crossover.

[0039] According to one embodiment of the present invention, the transformant may be a *Corynebacterium* sp. microorganism.

[0040] The *Corynebacterium* sp. microorganism may be, but is not limited to, *Corynebacterium glutamicum, Corynebacterium crudilactis, Corynebacterium deserti, Corynebacterium callunae, Corynebacterium suranareeae, Corynebacterium lubricantis, Corynebacterium doosanense, Corynebacterium efficiens, Corynebacterium uterequi, Corynebacterium stationis, Corynebacterium pacaense, Corynebacterium singulare, Corynebacterium humireducens, Corynebacterium marinum, Corynebacterium halotolerans, Corynebacterium spheniscorum, Corynebacterium freiburgense, Corynebacterium striatum, Corynebacterium canis, Corynebacterium ammoniagenes, Corynebacterium renale, Corynebacterium pollutisoli, Corynebacterium imitans, Corynebacterium caspium, Corynebacterium testudinoris, Corynebacaterium pseudopelargi*, or *Corynebacterium flavescens*.

[0041] The transformant in the present invention may be a strain either comprising the above-described phosphoenolpyruvate carboxylase variant or a polynucleotide encoding the same or comprising the vector comprising the same, a strain expressing the phosphoenolpyruvate carboxylase variant or the polynucleotide, or a strain having activity for the phosphoenolpyruvate carboxylase variant, without being limited thereto.

[0042] The transformant of the present invention may comprise other protein variants or genetic mutations, in addition to the phosphoenolpyruvate carboxylase variant.

[0043] According to one embodiment of the present invention, the transformant may have the ability to produce 5'-inosinic acid.

[0044] The 5'-inosinic acid is a nucleic acid-based compound that gives flavor to food, especially umami (savory) taste, and is used with the same meaning as inosine monophosphate (IMP).

[0045] The transformant may naturally have the ability to produce 5'-inosinic acid or may be one artificially endowed with the ability to produce 5'-inosinic acid.

[0046] According to one embodiment of the present invention, the transformant may have an increased ability to produce 5'-inosinic acid, due to a change in phosphoenolpyruvate carboxylase activity.

[0047] As used in the present invention, the term "increased ability to produce" means that 5'-inosinic acid productivity has increased compared to that of the parent strain. As used herein, the term "parent strain" refers to a wild-type strain or mutant strain to be mutated, and includes a strain that is to be mutated directly or to be transformed with a recombinant vector or the like. In the present invention, the parent strain may be a wild-type *Corynebacterium* sp. strain or a *Corynebacterium* sp. strain mutated from the wild-type microorganism.

[0048] The transformant according to the present invention exhibits an increased ability to produce 5'-inosinic acid compared to the parent strain, due to the change in phosphoenolpyruvate carboxylase activity caused by introduction of the phosphoenolpyruvate carboxylase variant thereinto, More specifically, the amount of 5'-inosinic acid produced by the transformant may be at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% higher than that produced by the parent strain, or may be 1.1-fold, 1.5-fold, 2-fold, 2,5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, or 10-fold higher than that produced by the parent strain, without being limited thereto. For example, the amount of 5'-inosinic acid produced by the transformant comprising the phosphoenolpyruvate carboxy-

lase variant may be at least 5%, specifically 5 to 50% (preferably 10 to 40%) higher than that produced by the parent strain.

[0049] Still yet another aspect of the present invention provides a method for producing 5'-inosinic acid, comprising steps of: culturing the transformant in a medium; and recovering 5'-inosinic acid from the transformant or the medium in which the transformant has been cultured.

[0050] The culturing may be performed using a suitable medium and culture conditions known in the art, and any person skilled in the art may easily adjust and use the medium and the culture conditions, Specifically, the medium may be a liquid medium, without being limited thereto. Examples of the culturing method include, but are not limited to, batch culture, continuous culture, fed-batch culture, or a combination thereof.

[0051] According to one embodiment of the present invention, the medium should meet the requirements of a specific strain in a proper manner, and may be appropriately modified by a person skilled in the art. For culture media for *Escherichia* sp. strains, reference may be made to, but (Manual of Methods for not limited to, a known document General Bacteriology, American Society for Bacteriology, Washington D.C., USA, 1981).

[0052] According to one embodiment of the present invention, the medium may contain various carbon sources, nitrogen sources, and trace element components. Examples of carbon sources that may be used include: sugars and carbohydrates such as glucose, sucrose, lactose, fructose, maltose, starch, and cellulose; oils and fats such as soybean oil, sunflower oil, castor oil, and coconut oil; fatty acids such as palmitic acid, stearic acid, and linoleic acid; alcohols such as glycerol and ethanol; and organic acids such as acetic acid. These substances may be used individually or as a mixture, without being limited thereto. Examples of nitrogen sources that may be used include peptone, yeast extract, meat extract, malt extract, Corn steep liquor, soybean meal, urea, or inorganic compounds such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate. The nitrogen sources may also be used individually or as a mixture, without being limited thereto. Examples of phosphorus sources that may be used include, but are not limited to, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts. In addition, the culture medium may contain, but is not limited to, metal salts such as magnesium sulfate or iron sulfate, which are required for growth. In addition, the culture medium may contain essential growth substances such as amino acids and vitamins. Moreover, suitable precursors may be used in the culture medium. The medium or individual components may be added to the culture medium batchwise or in a continuous manner by a suitable method during culturing, without being limited thereto.

[0053] According to one embodiment of the present invention, the pH of the culture medium may be adjusted by adding compounds such as ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid and sulfuric acid to the microorganism culture medium in an appropriate manner during the culturing. In addition, during the culturing, foaming may be suppressed using an anti-foaming agent such as a fatty acid polyglycol ester. Additionally, to keep the culture medium in an aerobic condition, oxygen or an oxygen-containing gas (for example, air) may be injected into the culture medium. The temperature of the culture medium may be generally 20° C. to 45° C., for example, 25° C. to 40° C. The culturing may be continued until a desired amount of a useful substance is produced. For example, the culturing time may be 10 hours to 160 hours.

[0054] According to one embodiment of the present invention, in the step of recovering 5'-inosinic acid from the cultured transformant the medium in which the transformant has been cultured, the produced 5'-inosinic acid may be collected or recovered from the medium using a suitable method known in the art depending on the culture method. Examples of a method that may be used to recover the produced 5'-inosinic acid include, but are not limited to, centrifugation, filtration, extraction, spraying, drying, evaporation, precipitation, crystallization, electrophoresis, fractional dissolution (e.g., ammonium sulfate precipitation), chromatography (e.g., ion exchange, affinity, hydrophobicity and size exclusion), and the like.

[0055] According to one embodiment of the present invention, the step of recovering 5'-inosinic acid may be performed by centrifuging the culture medium at a low speed to remove biomass and separating the obtained supernatant through ion-exchange chromatography.

[0056] According to one embodiment of the present invention, the step of recovering 5'-inosinic acid may include a process of purifying the 5'-inosinic acid.

Advantageous Effects

[0057] The phosphoenolpyruvate carboxylase variant according to the present invention is obtained by substituting one or more amino acids in the amino acid sequence constituting phosphoenolpyruvate carboxylase to change the activity of the protein, and a recombinant microorganism comprising the phosphoenolpyruvate carboxylase variant is capable of efficiently producing 5'-inosinic acid.

BRIEF DESCRIPTION OF DRAWINGS

[0058] FIG. **1** shows the structure of a pk19msb plasmid according to one embodiment of the present invention.

MODE FOR INVENTION

[0059] Hereinafter, the present invention will be described in more detail. However, this description is merely presented by way of example to facilitate the understanding of the present invention, and the scope of the present invention is not limited by this exemplary description.

Example 1. Construction of Strain Expressing Phosphoenolpyruvate Carboxylase Variant

[0060] To evaluate the effect of a variant (SEQ ID NO: 2) having a substitution of serine(S) for proline (P) at position 472 and a substitution of aspartic acid (D) for glycine (G) at position 673 in the amino acid sequence of phosphoenolpyruvate carboxylase (SEQ 4) on the production of 5'-inosinic acid, the present inventors constructed a vector for expressing the phosphoenolpyruvate carboxylase variant and a strain into which the vector has been introduced.

1-1. Construction of Vector for Expression of Phosphoenolpyruvate Carboxylase Variant

[0061] Using the genomic DNA of wild-type *Corynebacterium stationis* ATCC6872 as a template, PCR was per-

formed using a primer pair of primers 1 and 2. The PCR fragment and a pK19msb plasmid (SEQ ID NO: 5) were treated with the restriction enzyme smaI (NEB) and ligated together using T4 ligase. The resulting plasmid was named pK_PC.

[0062] The PCR amplification was performed using Pfu PreMix (Bioneer) under the following conditions: denaturation at 95° C. for 5 min, and then 30 cycles, each consisting of 95° C. for 30 sec, 58° C. for 30 sec, and 72° C. for 1 min and 30 sec, followed by reaction at 72° C. for 5 min.

[0063] The primer sequences used for plasmid construction are shown in Table 1 below,

TABLE 1

Primer name	SEQ ID NO.	Primer sequence (5'-3')
Primer 1	6	CTCTGCTCGCTCACTGGTCT
Primer 2	7	TACTCGCTGTGTCGTAGCGGG

1-2. Construction of Mutant Strain into Which Phosphoenolpyruvate Carboxylase Variant Has Been Introduced

[0064] An electrocompetent cell preparation method, a modification of the method of van der Rest et al., was used as a method for transformation of *Corynebacterium stationis* KCCM13339P.

[0065] First, *Corynebacterium stationis* KCCM13339P was primarily cultured in 10 mL of 2YT medium (containing 16 g/l of tryptone, 10 g/l of yeast extract, and 5 g/l of sodium chloride) supplemented with 2% glucose, thus preparing a seed culture. Isonicotinic acid hydrazine at a concentration of 1 mg/ml and 2.5% glycine were added to 100 ml of 2YT medium free of glucose. Next, the seed culture was inoculated into the 2YT medium to reach an OD_{610} value of 0.3, and then cultured at 30° C. and 180 rpm for 5 to 8 hours so that the OD_{610} value reached 0.6 to 0.7. The culture was kept on ice for 30 minutes, and then centrifuged at 3,500 rpm at 4° C. for 10 minutes. Thereafter, the supernatant was discarded and the precipitated *Corynebacterium stationis* KCCM13339P was washed 4 times with a 10% glycerol solution and finally re-suspended in 0.5 ml of a 10% glycerol solution, thereby preparing competent cells. Electroporation was performed using a Bio-Rad electroporator. The prepared competent cells and the constructed pK_PC vector were placed in an electroporation cuvette (0.2 mm), and then subjected to electroporation under conditions of 2.5 kV, 200Ω and 12.5 μF. Immediately after completion of the electroporation, 1 ml of regeneration (RG) medium (containing 18.5 g/l brain heart infusion and 0.5 M sorbitol) was added to the cells which were then heat-treated at 46° C. for 6 minutes. Next, the cells were cooled at room temperature, transferred into a 15-ml cap tube, incubated at 30° C. for 2 hours, and plated on a selection medium (containing 5 g/l tryptone, 5 g/l NaCl, 2.5 g/l yeast extract, 18.5 g/l brain heart infusion powder, 15 g/l agar, 91 g/l sorbitol, and 20 μg/l kanamycin). The cells were cultured at 30° C. for 72 hours, and the generated colonies were cultured in medium until the stationary phase to induce secondary recombination. Then, the cells were diluted to 10-5 to 10-7, and plated on an antibiotic-free plate medium (containing 10% sucrose), and a strain having no kanamycin resistance and grown on the medium containing 10% sucrose was selected and named IPC-1.

Experimental Example 1. Evaluation of 5'-Inosinic Acid Productivity of Strain Expressing Phosphoenolpyruvate Carboxylase Variant

[0066] 5'-Inosinic acid productivity was compared between the parent strain KCCM13339P and the mutant strain IPC-1 into which the phosphoenolpyruvate carboxylase variant has been introduced.

[0067] Each strain (parent strain or mutant strain) was inoculated at 1% by volume into a 100-mL flask containing 10 mL of the medium for 5'-inosinic acid production shown in Table 2 below, and cultured with shaking at 200 rpm at 34° C. for 45 hours. After completion of the culturing, the concentration of 5'-inosinic acid in the medium was measured using HPLC (Agilent), and the results are shown in Table 3 below.

TABLE 2

Component	Content
Glucose	70 g/L
$(NH_4)_2SO_4$	2 g/L
$MgSO_4$	1 g/L
Urea	2 g/L
Yeast extract	20 g/L
KH_2PO_4	2 g/L
$FeSO_4$	10 mg/L
$MnSO_4$	10 mg/L
Thiamine_HCl	5 mg/L
biotin	20 ug/L
Cystein	20 mg/L
Bata-alanine	20 mg/L
Adenine	30 mg/L

TABLE 3

Strain	5'-inosinic acid production (g/L)
KCCM13339P	20.0
IPC-1	23.2

[0068] As shown in Table 3 above, it was confirmed that the amount of 5'-inosinic acid produced by the mutant strain into which the phosphoenolpyruvate carboxylase variant has been introduced was increased by about 16% compared to that produced by the parent strain, due to substitution of the amino acids at positions 472 and 673 with other amino acids. These results suggest that introduction of point mutations into phosphoenolpyruvate carboxylase provides a significant effect on 5'-inosinic acid productivity.

[0069] So far, the present invention has been described with reference to the preferred embodiments. Those of ordinary skill in the art to which the present invention pertains will appreciate that the present invention may be embodied modified in forms without departing from the essential characteristics of the present invention. Therefore, the disclosed embodiments should be considered from an illustrative point of view, not from a restrictive point of view. The scope of the present invention is defined by the claims rather than the foregoing description, and all differences within the scope equivalent thereto should be construed as being included in the present invention

[Accession Number]

[0070] Depository Authority: Korean Culture Center of Microorganisms (KCCM)

[0071] Accession Number: KCCM13339P

[0072] Deposit Date: Mar. 29, 2023

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

INTERNATIONAL FORM

To: DAESANG
178 Magokjungang-ro,
Gangseo-gu,
Seoul, 07789,
Republic of Korea

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

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Corynebacterium stationis* MW 5520	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM13339P
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 April. 01. 1993 (date of the original deposit).[1] (KFCC-11786)	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on April. 01. 1993 (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on March. 29. 2023 (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Culture Center of Microorganisms Address: Yurim B/D 45, Hongjenae-2ga-gil Seodaemun-gu SEOUL 03461 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: June 29, 2022.

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

The above translation proves that there is no difference from the content of the original text.

Aguest 31, 2023

Patent Attorney Hyuk Park

SEQUENCE LISTING

Sequence total quantity: 7

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FEATURE Location/Qualifiers

source 1..2805

mol_type = other DNA

organism = synthetic construct

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organism = synthetic construct

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atttatttat tctgcgaagt gatcttccgt cacaggtatt tattcggcgc aaagtgcgtc 3960

-continued

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cgataagcta gcttcacgc                                               5719

SEQ ID NO: 6           moltype = DNA  length = 20
FEATURE                Location/Qualifiers
source                 1..20
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 6
ctctgctcgc tcactggtct                                             20

SEQ ID NO: 7           moltype = DNA  length = 21
FEATURE                Location/Qualifiers
source                 1..21
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 7
tactcgctgt gtcgtagcgg g                                           21
```

1. A phosphoenolpyruvate carboxylase variant in which one or more of the amino acids at positions 472 and 673 in the amino acid sequence of SEQ ID NO: 4 is/are substituted with other amino acid(s).

2. The phosphoenolpyruvate carboxylase variant of claim **1**, which consists of the amino acid sequence of SEQ ID NO: 2 in which proline at position 472 in the amino acid sequence of SEQ ID NO: 4 is substituted with serine and glycine at position 673 is substituted with aspartic acid.

3. A polynucleotide encoding the variant of claim **1**.

4. A transformant comprising the variant of claim **1**.

5. The transformant of claim **4**, which is a *Corynebacterium* sp. microorganism.

6. The transformant of claim **4**, which has ability to produce 5'-inosinic acid.

7. A method for producing 5'-inosinic acid, comprising steps of:

culturing the transformant of claim **4** in a medium; and

recovering 5'-inosinic acid from the transformant or the medium in which the transformant has been cultured.

8. A transformant comprising the polynucleotide of claim **3**.

* * * * *