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Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3095659 C 2022/12/06

(11)(21) 3 095 659

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 2017/10/20

(41) Mise à la disp. pub./Open to Public Insp.: 2018/07/05

(45) Date de délivrance/Issue Date: 2022/12/06

(62) Demande originale/Original Application: 3 048 802

(30) Priorité/Priority: 2016/12/28 (KR10-2016-0181343)

(51) Cl.Int./Int.Cl. *C12P 13/06* (2006.01), *C12N 1/21* (2006.01), *C12N 15/54* (2006.01), *C12N 15/67* (2006.01), *C12N 15/74* (2006.01), *C12N 9/10* (2006.01)

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(54) Titre : VARIANTE DE SYNTHASE D'ISOPROPYLMALATE ET METHODE DE PRODUCTION DE L-LEUCINE L'UTILISANT

(54) Title: ISOPROPYLMALATE SYNTHASE VARIANT AND A METHOD OF PRODUCING L-LEUCINE USING THE SAME

(57) Abrégé/Abstract:
The present disclosure relates to a novel modified polypeptide having an isopropylmalate synthase activity, a polynucleotide encoding the same, a microorganism comprising the polypeptide, and a method of producing L-leucine by culturing the microorganism.

[ABSTRACT]

[Abstract]

The present disclosure relates to a novel modified polypeptide having an isopropylmalate synthase activity, a polynucleotide encoding the same, a microorganism comprising the polypeptide, and a method of producing L-leucine by culturing the microorganism.

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[DESCRIPTION]

[Invention Title]

Isopropylmalate Synthase Variant and a Method of Producing L-Leucine Using the Same

[Technical Field]

The present disclosure relates to a novel modified polypeptide having an isopropylmalate synthase activity, a polynucleotide encoding the same, a microorganism comprising the polypeptide, and a method of producing L-leucine by culturing the microorganism.

[Background Art]

L-Leucine is an essential amino acid, one which is expensive and widely used in medicaments, foods, feed additives, industrial chemicals, *etc*. In addition, L-leucine is mainly produced using a microorganism. Fermentation of branched-chain amino acids including L-leucine is mainly carried out through a microorganism of the genus *Escherichia* or a microorganism of the genus *Corynebacterium*, known to biosynthesize 2-ketoisocaproate as a precursor from pyruvic acid though several steps (Korean Patent Nos. 10-0220018 and 10-0438146).

Isopropylmalate synthase (hereinafter referred to as "IPMS"), which is an enzyme involved in the biosynthesis of leucine, is an enzyme of the first step in the biosynthesis of leucine, which converts 2-ketoisovalerate, produced during the valine biosynthetic pathway, to isopropylmalate, allowing the biosynthesis of leucine instead of valine, and thereby IPMS is an important enzyme in the process of leucine biosynthesis. However, the IPMS is subject to feedback inhibition by L-leucine, which is a final product, or derivatives thereof. Accordingly, although there is a variety of prior art relevant to IPMS variants which release feedback inhibition for the purpose of producing a high concentration of leucine (U.S. Patent Publication Application No. 2015-0079641 and U.S. Patent No. 6403342), research is still continuing to discover better variants.

[Disclosure]

[Technical Problem]

The present inventors have endeavored to develop an IPMS variant that can be used for

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the production of L-leucine with a high concentration, and as a result, the present inventors developed a novel IPMS variant. It was confirmed that the variant released feedback inhibition by L-leucine, which is a final product, and enhanced an activity thereof such that the variant is capable of producing L-leucine at a high yield from a microorganism containing the same, thereby completing the present disclosure.

[Technical Solution]

An object of the present disclosure is to provide a novel modified polypeptide having an isopropylmalate synthase activity.

Another object of the present disclosure is to provide a polynucleotide encoding the modified polypeptide.

Still another object of the present disclosure is to provide a microorganism of the genus *Corynebacterium* producing L-leucine, containing the polypeptide.

Still another object of the present disclosure is to provide a method of producing L-leucine by culturing the microorganism in a medium.

[Advantageous Effects]

The novel modified polypeptide having an activity of isopropylmalate synthase is a polypeptide in which the activity is increased compared to that of the wild-type and feedback inhibition by L-leucine is released, and thereby L-leucine can be produced in a high yield using such modified polypeptide.

[Best Mode for Carrying Out the Invention]

To achieve the above objects, an aspect of the present disclosure provides a novel modified polypeptide having an isopropylmalate synthase activity. The novel modified polypeptide may be a modified polypeptide having an isopropylmalate synthase activity, wherein arginine at position 558 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 is substituted with an amino acid residue other than arginine, or glycine at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 is substituted with an amino acid residue other than glycine. The modified polypeptide of the present disclosure not only has an activity higher than that of a polypeptide of SEQ ID NO: 1 having an isopropylmalate synthase activity, but also has a feature

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that feedback inhibition by L-leucine is released.

As used herein, the term "isopropylmalate synthase" refers to an enzyme converting 2-ketoisovalerate to isopropylmalate, which is a precursor of L-leucine, by reacting with acetyl-CoA. The isopropylmalate synthase of the present disclosure may be included as long as the enzyme has the conversion activity, regardless of an origin of a microorganism. Specifically, the isopropylmalate synthase may be an enzyme derived from a microorganism of the genus *Corynebacterium*. More specifically, the isopropylmalate synthase may be an isopropylmalate synthase derived from *Corynebacterium glutamicum*, and specifically, it may include the amino acid sequence of SEQ ID NO: 1, but is not limited thereto. Additionally, the isopropylmalate synthase may include a polypeptide having homology of at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% with the amino acid sequence of SEQ ID NO: 1. For example, it is obvious that an amino acid sequence having such homology and exhibiting an effect corresponding to that of the isopropylmalate synthase can be included within the scope of the present disclosure even if it has an amino acid sequence in which some of the sequences are deleted, modified, substituted, or added.

As used herein, the term "increase in activity of isopropylmalate synthase" refers to an increase in the conversion activity to isopropylmalate. Therefore, the modified polypeptide of the present disclosure has a higher level of the isopropylmalate conversion activity compared to a polypeptide of SEQ ID NO: 1 having the activity of isopropylmalate synthase. The isopropylmalate conversion activity can be directly confirmed by measuring the level of isopropylmalate produced, or can be indirectly confirmed by measuring the level of CoA produced. As used herein, the term "increase in activity" may be used in combination with "enhanced activity". Further, isopropylmalate is a precursor of L-leucine, and thus the use of the modified polypeptide of the present disclosure results in producing a higher level of L-leucine compared to a polypeptide of SEQ ID NO: 1 having the activity of isopropylmalate synthase.

Additionally, unlike a polypeptide of SEQ ID NO: 1 having the activity of isopropylmalate synthase, the modified polypeptide of the present disclosure may be characterized in that feedback inhibition by L-leucine, which is a final product, or a derivative thereof is released. As used herein, the term "feedback inhibition" refers to the inhibition of a reaction at the early state of an enzyme system by a final product in the enzyme system. For the objects of the present disclosure, the feedback inhibition may be feedback inhibition in which

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L-leucine or a derivative thereof inhibits the activity of isopropylmalate synthase, which mediates the first step of the biosynthetic pathway, but is not limited thereto. Therefore, when the feedback inhibition of isopropylmalate synthase is released, the productivity of L-leucine can be increased compared with the case of not releasing the same.

As used herein, the term "modification", "modified", or "variant" refers to a culture or an individual that shows an inheritable or non-heritable alternation in one stabilized phenotype. Specifically, the term "variant" may be intended to mean a variant in which its activity is efficiently increased because the amino acid sequence corresponding to *Corynebacterium glutamicum*-derived isopropylmalate synthase is modified compared to the wild-type, a variant in which feedback inhibition by L-leucine or a derivative thereof is released, or a variant in which the increase in activity and feedback inhibition are both released.

Specifically, the modified polypeptide of the present disclosure, which has the activity of isopropylmalate synthase, may be a modified polypeptide having an activity of isopropylmalate synthase, wherein arginine, an amino acid at position 558 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with an amino acid residue other than arginine, or glycine, an amino acid residue at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with an amino acid residue other than glycine. The amino acid other than arginine may include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, histidine, aspartic acid, and glutamic acid; and the amino acid other than glycine may include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, methionine, arginine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, histidine, aspartic acid, and glutamic acid; but the amino acids are not limited thereto. More specifically, the modified polypeptide may be a modified polypeptide, wherein arginine, an amino acid residue at position 558 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with histidine, alanine, or glutamine; or glycine, an amino acid residue at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with aspartic acid, arginine, or tyrosine, but is not limited thereto. Additionally, the modified polypeptide may be one in which the arginine at position 558 is substituted with histidine, alanine, or glutamine; and the glycine at position 561 is substituted with aspartic acid, arginine, or tyrosine, but is not

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limited thereto. Most specifically, the modified polypeptide may include an amino acid sequence of any one of SEQ ID NO: 21 to SEQ ID NO: 35.

Additionally, the modified polypeptide may include a polypeptide having homology of at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% with the amino acid sequence of any one of SEQ ID NO: 21 to SEQ ID NO: 35. For example, it is obvious that an enzyme variant having an amino acid sequence, in which some of the sequences are deleted, modified, substituted, or added while the modified amino acid sequence corresponding to the amino acid sequence at positions 558 and/or 561 is fixed, should also belong to the scope of the present disclosure as long as the amino acid sequence has the homology above and exhibits an effect corresponding to that of isopropylmalate synthase. On the other hand, positions 558 and 561, which are specific modification positions, refer to positions that are determined based on the N-terminus in the amino acid sequence of SEQ ID NO: 1, and therefore, the fact that such positions are determined by considering the number of the amino acids which are added to or deleted from the N-terminus of SEQ ID NO: 1 is obvious to one of ordinary skill in the art, and thereby also belongs to the scope of the present disclosure. For example, leuA, which is the gene encoding isopropylmalate synthase, was represented by SEQ ID NO: 1 consisting of 616 amino acids. However, in some references, the translation initiation codon is indicated 35 amino acids downstream of the sequence of the leuA gene, *i.e.*, a gene consisting of 581 amino acids. In such a case, the 558^{th} amino acid is interpreted as the 523^{rd} amino acid and the 561^{st} amino acid as the 526^{th} amino acid, and is thereby included in the scope of the present disclosure.

As used herein, the term "homology" refers to a percentage of identity between two polynucleotides or polypeptide moieties. The homology between sequences from a moiety to another moiety may be determined by the technology known in the art. For example, the homology may be determined by directly arranging the sequence information, *i.e.*, parameters such as score, identity, similarity, *etc.*, of two polynucleotide molecules or two polypeptide molecules using an easily accessible computer program (Example: BLAST 2.0). Additionally, the homology between polynucleotides may be determined by hybridizing polynucleotides under the condition of forming a stable double-strand between the homologous regions, disassembling with a single strand-specific nuclease, followed by size determination of the disassembled fragments.

Another aspect of the present disclosure provides a polynucleotide encoding the modified polypeptide.

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The polynucleotide may be a polynucleotide encoding a modified polypeptide having the activity of isopropylmalate synthase, wherein arginine, an amino acid at position 558 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with another amino acid residue other than arginine, or glycine, an amino acid residue at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1, is substituted with another amino acid residue other than glycine. Specifically, a polynucleotide encoding a polypeptide including the amino acid sequence of SEQ ID NOs: 21 to 35 and having an activity of isopropylmalate synthase; a modified polypeptide having homology of at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% with the polypeptide above; or encoding a modified polypeptide having an activity of isopropylmalate synthase, in which some of the sequences are deleted, modified, substituted, or added while the modified amino acid sequence at positions 558 and/or 561, which are specific modification positions in the polypeptide above, is fixed may be included without limitation. Alternately, the probe that can be prepared from a known gene sequence, for example, a sequence encoding a protein having activity of Isopropylmalate synthase by hybridization of a complementary sequence for all or part of the nucleotide sequence above under stringent conditions, can be included without limitation.

As used herein, the term "stringent conditions" refers to conditions under which a so-called hybrid is formed while non-specific hybrids are not formed. Examples of such conditions include conditions under which genes having high degrees of homology, such as genes having a homology of 80% or more, specifically 90% or more, more specifically 95% or more, furthermore specifically 97% or more, and most specifically 99% or more, hybridize with each other while genes having low degrees of a homology do not hybridize with each other, or conditions under which genes are washed 1 time, and specifically 2 and 3 times, at a temperature and a salt concentration equivalent to 60°C, 1×SSC, and 0.1% SDS, specifically 60°C, 0.1×SSC, and 0.1% SDS, and more specifically 68°C, 0.1×SSC, and 0.1% SDS, which are the conditions for washing of ordinary Southern hybridization (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001)).

The probe used in the hybridization may be a part of the complementary sequence of the nucleotide sequence. Such probe can be constructed by PCR using an oligonucleotide prepared based on a known sequence as a primer and a gene fragment containing such nucleotide

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sequence as a template. For example, a gene fragment having a length of about 300 bp can be used as a probe. More specifically, in the case of using a probe having a length (about 300 bp), 50°C, 2×SSC, and 0.1% SDS may be suggested for the washing conditions of hybridization.

On the other hand, the polynucleotide may be a polynucleotide having a nucleotide sequence of any one of SEQ ID NO: 36 to SEQ ID NO: 50, and it is obvious that the polynucleotide also includes a polynucleotide that can be translated into the modified polypeptide by codon degeneracy.

Still another aspect of the present disclosure is to provide a microorganism of the genus *Corynebacterium* producing L-leucine, containing the modified polypeptide.

In the present disclosure, the microorganism may include all of a microorganism artificially produced through transformation or a naturally-occurring microorganism.

As used herein, the term "transformation" refers to the introduction of a gene into a host cell for expression. In the present disclosure, the transformation method includes any method that introduces a gene into a cell and can be carried out by selecting a suitable standard technique known in the art. Examples of the transformation method are electroporation, calcium phosphate co-precipitation, retroviral infection, microinjection, DEAE-dextran, cationic liposome, heat shock method, *etc.*, but are not limited thereto.

The gene to be transformed may include both a form inserted into the chromosome of a host cell and a form located outside the chromosome, as long as it can be expressed in the host cell. In addition, the gene includes DNA and RNA as a polynucleotide capable of encoding a polypeptide, and any gene that can be introduced and expressed in the host cell can be used without limitation. For example, the gene can be introduced into a host cell in a form of an expression cassette, which is a polynucleotide construct containing all elements required for self-expression. The expression cassette usually includes a promoter operably linked to the gene, a transcription termination signal, ribosome binding sites, and a translation termination signal. The expression cassette may be in a form of a self-replicable expression vector. In addition, the gene may be one introduced into a host cell itself or in a form of a polynucleotide construct, *i.e.*, a form of a vector, and operably linked to the sequences required for expression in the host cell.

As used herein, the term "vector" refers to any carrier for cloning and/or transferring

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nucleotides to a host cell. A vector may be a replicon to allow for the replication of the fragments combined with other DNA fragments. "Replicon" refers to any genetic unit acting as a self-replicating until for DNA replication *in vivo*, that is, replicable by self-regulation (*e.g.*, plasmid, phage, cosmid, chromosome, and virus). The term "vector" may include viral and non-viral carriers for introducing nucleotides into a host cell *in vitro*, *ex vivo*, or *in vivo*, and may also include a mini-spherical DNA. For example, the vector may be a plasmid without a bacterial DNA sequence. Removal of bacterial DNA sequences which are rich in CpG area has been conducted to reduce silencing of the transgene expression and to promote more continuous expression from a plasmid DNA vector (for example, Ehrhardt, A. *et al.* (2003) Hum Gene Ther 10: 215-25; Yet, N. S. (2002) MoI Ther 5: 731-38; Chen, Z. Y. *et al.* (2004) Gene Ther 11 : 856-64). The term "vector" also may include a transposon such as Sleeping Beauty (Izsvak *et al.* J. Mol. Biol. 302:93-102 (2000)), or an artificial chromosome. Examples of the vector typically used may be natural or recombinant plasmid, cosmid, virus, and bacteriophage. For example, as the phage vector or the cosmid vector, pWE15, M13, λMBL3, λMBL4, λIXII, λASHII, λAPII, λt10, λt11, Charon4A, Charon21A, *etc.* may be used. In addition, as the plasmid vector, pDZ type, pBR type, pUC type, pBluescriptII type, pGEM type, pTZ type, pCL type, pET type, *etc.* may be used. Specifically, pECCG117 vector may be used. The vector that can be used in the present disclosure is not particularly limited, and the known expression/substitution vector may be used.

In addition, the vector may be a recombinant vector which may further include various antibiotic resistance genes.

As used herein, the term "antibiotic resistance gene" refers to a gene having resistance to antibiotics, and the cells comprising this gene survive even in the environment treated with the corresponding antibiotic. Therefore, the antibiotic resistance gene can be effectively used as a selection marker for a large-scale production of plasmids in microorganisms, such as *E. coli*, *etc.* In the present invention, as the antibiotic resistance gene is not a factor that significantly affects the expression efficiency which is obtained by an optimal combination of components of the vector which is the key feature of the present invention, any common antibiotic resistance gene can be used as a selection marker without limitation. Specifically, the resistance genes against ampicilin, tetracyclin, kanamycin, chloramphenicol, streptomycin, or neomycin can be used.

As used herein, the term "operably linked" refers to the operable linking of a regulatory sequence for nucleotide expression with a nucleotide sequence encoding a target protein for

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performing its general function, thereby affecting the expression of a coding nucleotide sequence. Operable linking with a vector can be made using a gene recombination technique known in the art, and site-specific DNA cleavage and ligation can be performed using a restriction enzyme and ligase known in the art.

As used herein, the term "host cell in which a vector is introduced (transformed)" refers to a cell transformed with a vector having a gene encoding one or more target proteins. The host cell may include any of a prokaryotic microorganism and a eukaryotic microorganism as long as the microorganism includes a modified polypeptide capable of producing isopropylmalate synthase by introducing the vector above. For example, the microorganism strain belonging to the genera of *Escherichia*, *Erwinia*, *Serratia*, *Providencia*, *Corynebacterium*, and *Brevibacterium* may be included. An example of the microorganism of the genus *Corynebacterium* may be *Corynebacterium glutamicum*, but is not limited thereto.

The microorganism of the genus *Corynebacterium* producing L-leucine, which is capable of expressing the modified polypeptide having the activity of isopropylmalate synthase, includes all microorganisms capable of expressing the modified polypeptide by various known methods in addition to the introduction of a vector.

Still another aspect of the present disclosure provides a method of producing L-leucine, comprising: (a) culturing the microorganism of the genus *Corynebacterium* producing L-leucine; and (b) recovering L-leucine from the cultured microorganism or the cultured medium.

As used herein, the term "culture" refers to culturing of the microorganism under appropriately controlled environmental conditions. The culturing process of the present disclosure may be carried out depending on a suitable medium and culture condition known in the art. Such culturing process can be easily adjusted and used by one of ordinary skill in the art depending on the strain to be selected. Specifically, the culture may be a batch type, a continuous type, and a fed-batch type, but is not limited thereto.

The carbon sources contained in the medium may 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, coconut oil, *etc.*; 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 materials may be used alone or in combinations thereof, but are not limited thereto.

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The nitrogen sources contained in the medium may include organic nitrogen sources, such as peptone, yeast extract, gravy, malt extract, corn steep liquor, and soybean; and inorganic nitrogen sources, such as urea, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate. These nitrogen sources may be used alone or in combinations thereof, but are not limited thereto. The phosphorous sources contained in the medium may include potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and corresponding sodium-containing salts, but are not limited thereto. Additionally, metal salts such as magnesium sulfate or iron sulfate may be contained. In addition, amino acids, vitamins, suitable precursors, *etc*. may be contained. These media or precursors may be added in a batch culture process or a continuous culture process to a culture, but are not limited thereto.

pH of the culture may be adjusted during the cultivation by adding an appropriate compound such as ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid, and sulfuric acid, and the generation of foams may be inhibited during the cultivation by using an antifoaming agent such as fatty acid polyglycol ester. In order to maintain aerobic conditions of the culture, oxygen or oxygen-containing gas may be injected into the culture. In order to maintain anaerobic and microaerobic conditions, no gas may be injected or nitrogen, hydrogen, or carbon dioxide may be injected. The temperature of the culture may be 27°C to 37°C, and specifically 30°C to 35°C, but is not limited thereto. The period of cultivation may be continued as long as the desired amount of useful material is recovered, and preferably for 10 to 100 hours, but the period of cultivation is not limited thereto.

The step of recovering L-leucine produced in the culture step of the present disclosure can collect the desired L-leucine from the microorganism or the medium using a suitable method known in the art depending on culture methods. For example, centrifugation, filtration, anion exchange chromatography, crystallization, HPLC, *etc*. may be used, and a suitable method known in the art may be used to recover the desired L-leucine from the medium or the microorganism. Additionally, the recovery step above may include a purification process.

[Mode for Carrying Out the Invention]

Hereinbelow, the present disclosure will be described in detail with accompanying exemplary embodiments. However, the exemplary embodiments disclosed herein are only for illustrative purposes and should not be construed as limiting the scope of the present disclosure.

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Example 1: Confirmation of leuA nucleotide sequence of KCCM11661P, microorganism producing leucine

Corynebacterium glutamicum ATCC14067 was inoculated into a seed medium having the ingredients described below at 121°C for 15 minutes, cultured for 13 hours, and then 25 mL of the culture medium was recovered. The recovered culture medium was washed with a 100 mM citrate buffer and treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) for 30 minutes to a final concentration of 400 μg/mL. Thereafter, the resultant was washed with a 100 mM phosphate buffer. The mortality rate of the strains treated with NTG was determined to be 99.6% as a result of smearing the strains on a minimal medium having the ingredients described below. In order to achieve variants resistant to norleucine (NL), the NTG-treated strains were smeared on the minimal media with final concentrations of 20 mM, 40 mM, and 50 mM, cultured at 30°C for 5 days, and then variants resistant to NL were obtained.

<Seed medium>

Glucose (20 g), peptone (10 g), yeast extract (5 g), carbamide (1.5 g), KH_2PO_4 (4 g), K_2HPO_4 (8 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), biotin (100 μg), thiamine hydrochloride (1,000 μg), calcium-pantothenic acid (2,000 μg), nicotinamide (2,000 μg; based on 1 liter of distilled water), pH 7.0

<Production medium >

Glucose (100 g), $(NH_4)_2SO_4$ (40 g), soy protein (2.5 g), corn steep solid (5 g), urea (3 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), biotin (100 μg), thiamine hydrochloride (1,000 μg), calcium-pantothenic acid (2000 μg), nicotinamide (3,000 μg), $CaCO_3$ (30 g; based on 1 liter of distilled water), pH 7.0

The variants obtained by the method above were designated as *Corynebacterium glutamicum* KCJ-24 and *Corynebacterium glutamicum* KCJ-28 and deposited to the Korean Culture Center of Microorganisms, an international depositary authority, on January 22, 2015, under the Budapest Treaty, and as a result, *Corynebacterium glutamicum* KCJ-24 and *Corynebacterium glutamicum* KCJ-28 were deposited under Accession Nos. KCCM11661P and

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KCCM11662P, respectively. *Corynebacterium glutamicum* KCJ-24 and *Corynebacterium glutamicum* KCJ-28 produced L-leucine at a concentration of 2.7 g/L and 3.1 g/L, respectively. Therefore, it was confirmed that the productivity of L-leucine produced from the variants was 10-fold higher than that of the wild-type.

Additionally, an attempt was made to confirm whether the variation of leuA encoding isopropylmalate synthase (IPMS) occurred in the variant KCCM11661P. The amino acid sequence (SEQ ID NO: 1) of wild-type leuA was confirmed by referring to WP_003863358.1 of Genebank. The chromosomal DNA of the variant was amplified using a polymerase chain reaction (hereinafter referred to as 'PCR') method. Although it is known that the leuA gene consists of 616 amino acids, in some references, it is published that the translation initiation codon is indicated 35 amino acids downstream of the sequence of the leuA gene, and thereby the leuA gene consists of 581 amino acids. In such a case, the position number indicating the variation of the corresponding amino acid can vary. Therefore, in cases where the leuA gene is considered to consist of 581 amino acids, the variation position is additionally indicated in parenthesis.

Specifically, PCR was performed using the chromosomal DNA of the variant as a template and using primers of SEQ ID NOs: 3 and 4 under the following conditions: denaturation at 94°C for 1 minute; annealing at 58°C for 30 seconds; and polymerization at 72°C for 2 minutes using Taq DNA polymerase. Such PCR was repeated a total of 28 times to amplify a fragment of about 2,700 base pairs. The nucleotide sequence of the fragment was analyzed using the same primer, and as a result, it was confirmed that G, which is the 1673nd nucleotide of leuA in KCCM11661P, was substituted with A. This result implies that arginine, which is the 558th (or 523rd; hereinafter only indicated as 558th) amino acid, is substituted with histidine. In addition, it was also confirmed that GC, which are the 1682nd and 1683rd nucleotides, were substituted with AT. This result also implies that glycine, which is the 561st (or 526th, hereinafter only indicated as 561st) amino acid, is substituted with aspartic acid.

Example 2: Production of substitution vector of IPMS variant

In order to produce a vector containing the modified nucleotide sequence confirmed in Example 1, PCR was performed using the chromosomal DNA of the variant above as a template

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and using primers of SEQ ID NOs: 5 and 6 under the following conditions: denaturation at 94°C for 1 minute; annealing at 58°C for 30 seconds; and polymerization at 72°C for 1 minute using Pfu DNA polymerase. Such PCR was repeated a total of 25 times to amplify a fragment of about 1,460 base pairs with SalI and XbaI restriction enzyme sites. The amplified fragment was treated with restriction enzymes, SalI and XbaI, and then pDZ-leuA (R558H, G561D) was prepared by ligation with the vector pDZ (Korean Patent No: 10-0924065 and International Patent Publication No. 2008-033001) treated with the same enzymes. Additionally, in order to prepare a vector with each variation, ATCC14067 was used as a template, and then 2 fragments were amplified using primers 5 and 7, and primers 8 and 6, respectively. PCR was performed using the two prepared fragments as templates under the following conditions: denaturation at 94°C for 1 minute; annealing at 58°C for 30 seconds; and polymerization at 72°C for 1 minute using Pfu DNA polymerase. Such PCR was repeated a total of 25 times to amplify a fragment of about 1,460 base pairs with SalI and XbaI restriction enzyme sites. The amplified fragment was treated with restriction enzymes SalI and XbaI, and then pDZ-leuA (R558H) was prepared by ligation with pDZ treated with the same enzymes. pDZ-leuA (G561D) was prepared using primers 5 and 9, and primers 10 and 6 by the same method above.

Example 3: Production of substitution strain of IPMS variant

Corynebacterium glutamicum ATCC14067 was used as a parent strain in order to prepare a strain containing the leuA-modified nucleotide sequence which was found in the modified strain above.

Corynebacterium glutamicum ATCC14067 was transformed with the vectors pDZ-leuA (R558H), pDZ-leuA (G561D), and pDZ-leuA (R558H, G561D), which were prepared in Example 2 by electroporation. Each of the strains prepared through the secondary crossover was designated as 14067::leuA (R558H), 14067::leuA (G561D), and 14067::leuA (R558H, G561D). In order to confirm whether the nucleotide of leuA was substituted, PCR was performed using primers of SEQ ID NOs: 3 and 4 under the following conditions: denaturation at 94°C for 1 minute; annealing at 58°C for 30 seconds; and polymerization at 72°C for 2 minutes using Taq DNA polymerase. Such PCR was repeated a total of 28 times to amplify a fragment of about 2,700 base pairs. Thereafter, the substitution of the nucleotide of leuA was

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confirmed by analyzing the nucleotide sequence with the same primer.

The strain, 14067::leuA (R558H, G561D) which was transformed with the vector pDZ-leuA (R558H, G561D), was designated as KCJ-0148, and deposited to the Korean Culture Center of Microorganisms on January 25, 2016, and as a result, the strain was deposited under Accession No. KCCM11811P.

Example 4: Production of L-leucine in substitution strain of IPMS variant

In order to produce L-leucine from *Corynebacterium glutamicum* 14067::leuA (R558H), 14067::leuA (G561D), and 14067::leuA (R558H, G561D), which were prepared in Example 3, cultivation was carried out in the following manner.

A platinum loop of each of the parent strain, *Corynebacterium glutamicum* ATCC14067, and the prepared *Corynebacterium glutamicum* 14067::leuA (R558H), 14067::leuA (G561D), and 14067::leuA (R558H, G561D) strains was inoculated into a corner-baffled flask (250 mL) containing a production medium (25 mL). Thereafter, L-leucine was produced by incubating in a shaking water bath at 30°C at a rate of 200 rpm for 60 hours.

After completion of the incubation, the amount of L-leucine produced was measured by high performance liquid chromatography. The concentration of L-leucine in the culture medium for each experimental strain is shown in Table 1 below.

[Table 1] Production of L-leucine in substitution strain of IPMS variant

Strain	L-Leucine concentration (g/L)
ATCC14067	0.1
14067::leuA (R558H)	1.2
14067::leuA (G561D)	1.6
14067::leuA (R558H, G561D)	2.5

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As shown in Table 1 above, it was confirmed that the L-leucine productivity of the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::leuA (R558H), 14067::leuA (G561D), and 14067::leuA (R558H, G561D), which have the R558H, G561D, or R558H/G561D variation in the leuA gene, was enhanced about 12- to 25-fold compared to that of the parent strain, *Corynebacterium glutamicum* ATCC14067.

Example 5: Production of IPMS variant-overexpressing vector

In order to produce an expression vector containing the modified nucleotide sequence confirmed in Example 1, PCR was carried out using ATCC14067 and the chromosomal DNA of the 3 variants prepared in Example 3 as templates and using primers of SEQ ID NOs: 11 and 12 under the following conditions: denaturation at 94°C for 1 minute; annealing at 58°C for 30 seconds; and polymerization at 72°C for 1 minute using Pfu DNA polymerase. Such PCR was repeated a total of 25 times to amplify a fragment of about 2,050 base pairs with NdeI and XbaI restriction enzyme sites. The amplified fragment was treated with restriction enzymes, NdeI and XbaI, and then expression vectors p117_PCJ7-leuA (WT), p117_PCJ7-leuA (R558H), p117_PCJ7-leuA (G561D), and p117_ PCJ7-leuA (R558H, G561D) were prepared by ligation using p117_PCJ7 in which a PCJ7 promoter was inserted in the vector pECCG117 (Biotechnology letters Vol. 13, No. 10, p. 721-726 (1991)) treated with the same enzymes. The PCJ7 promoter is a promoter that enhances gene expression, and is publicly known in Korean Patent No. 10-0620092 and International Patent Publication No. 2006-065095.

Example 6: Production of strain transformed with IPMS variant-overexpressing vector

In order to produce a strain transformed with an overexpression vector containing the leuA modified nucleotide sequence prepared in Example 5, the parent strain, which is wild-type *Corynebacterium glutamicum* ATCC14067, and the leucine-producing strains KCCM11661P and KCCM11662P were used.

Each of the vectors p117_PCJ7-leuA (WT), p117_PCJ7-leuA (R558H), p117_PCJ7-leuA (G561D), and p117_PCJ7-leuA (R558H, G561D), prepared in Example 5, was transformed with *Corynebacterium glutamicum* ATCC14067, KCCM11661P, and KCCM11662P by

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electroporation. As a result, 14067::p117_PCJ7-leuA (WT), 14067::p117_PCJ7-leuA (R558H), 14067::p117_PCJ7-leuA (G561D), 14067::p117_PCJ7-leuA (R558H,G561D); KCCM11661P::p117_PCJ7-leuA (WT), KCCM11661P::p117_PCJ7-leuA (R558H), KCCM11661P::p117_PCJ7-leuA (G561D), KCCM11661P::p117_PCJ7-leuA (R558H, G561D); and KCCM11662P::p117_PCJ7-leuA (WT), KCCM11662P::p117_PCJ7-leuA (R558H), KCCM11662P::p117_PCJ7-leuA (G561D), KCCM11662P::p117_PCJ7-leuA (R558H, G561D) were produced.

Example 7: Production of L-leucine in strain transformed with IPMS variant-overexpressing vector

In order to produce L-leucine from the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (WT), 14067::p117_PCJ7-leuA (R558H), 14067::p117_PCJ7-leuA (G561D), 14067::p117_PCJ7-leuA (R558H, G561D); KCCM11661P:: p117_PCJ7-leuA (WT), KCCM11661P::117PCJ7-leuA (R558H), KCCM11661P::p117_PCJ7-leuA (G561D), KCCM11661P::p117_PCJ7-leuA (R558H, G561D); and KCCM11662P::p117_PCJ7-leuA (WT), KCCM11662P::p117_PCJ7-leuA (R558H), KCCM11662P::p117_PCJ7-leuA (G561D), KCCM11662P::p117_PCJ7-leuA (R558H, G561D), which were produced in Example 6, cultivation was carried out in the following manner.

A platinum loop of each of the parent strains, *Corynebacterium glutamicum* ATCC14067, KCCM11661P, and KCCM11662P, and the strains produced in Example 6 was inoculated into a corner-baffled flask (250 mL) containing a production medium (25 mL). Thereafter, L-leucine was produced by incubating in a shaking water bath at 30°C at a rate of 200 rpm for 60 hours.

After completion of the incubation, the amount of L-leucine produced was measured by high performance liquid chromatography. The concentration of L-leucine in the culture medium for each experimental strain is shown in Table 2 below.

[Table 2] Production of L-leucine in strain overexpressing IPMS variant

Strain	L-Leucine

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	concentration (g/L)
ATCC14067	0.1
14067:: p117_PCJ7-leuA (WT)	0.3
14067:: p117_PCJ7-leuA (R558H)	4.5
14067::p117_PCJ7-leuA (G561D)	5.1
14067::p117_PCJ7-leuA (R558H,G561D)	9.8
KCCM11661P	2.7
KCCM11661P:: p117_PCJ7-leuA (WT)	3.0
KCCM11661P:: p117_PCJ7-leuA (R558H)	6.1
KCCM11661P::p117_PCJ7-leuA (G561D)	6.8
KCCM11661P::p117_PCJ7-leuA (R558H,G561D)	12.3
KCCM11662P	3.1
KCCM11662P:: p117_PCJ7-leuA (WT)	3.3
KCCM11662P:: p117_PJ7-leuA (R558H)	6.3
, KCCM11662P::p117_PCJ7-leuA (G561D)	6.9
KCCM11662P::p117_PCJ7-leuA (R558H,G561D)	13.1

As shown in Table 2 above, it was confirmed that the L-leucine production of the L-leucine-producing strains, 14067::p117_PCJ7-leuA (R558H), 14067::p117_PCJ7-leuA (G561D), and 14067::p117_PCJ7-leuA (R558H, G561D), which were transformed with the overexpression vector containing variation of the leuA gene in the strain ATCC14067, was enhanced 45- to 98-fold compared to that of the parent strain ATCC14067; the L-leucine production of the L-leucine-producing strains, KCCM11661P::p117_PCJ7-leuA (R558H), KCCM11661P::p117_PCJ7-leuA (G561D), and KCCM11661P::p117_PCJ7-leuA (R558H, G561D), which were transformed with the overexpression vector containing variation of the leuA gene in the strain KCCM11661P, was enhanced 2.3- to 4.5-fold compared to that of the parent strain KCCM11661P; and that the L-leucine production of the L-leucine-producing strains, KCCM11662P::p117_PCJ7-leuA (R558H), KCCM11662P::p117_PCJ7-leuA (G561D), and KCCM11662P::p117_PCJ7-leuA (R558H,G561D), which were transformed with the

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overexpression vector containing variation of the leuA gene in the strain KCKCM11662P, was enhanced 2- to 4.2-fold compared to that of the parent strain KCCM11662P.

Example 8: Measurement of isopropylmalate synthase activity in strain transformed with leuA-overexpressing vector

In order to measure an isopropylmalate synthase activity in the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (WT), 14067::p117_PCJ7-leuA (R558H), 14067::p117_PCJ7-leuA (G561D), and 14067::p117_PCJ7-leuA (R558H, G561D), produced in Example 6, experiments were carried out in the following manner.

A platinum loop of each of the 4 strains above was inoculated into a corner-baffled flask (250 mL) containing the seed medium (25 mL). Thereafter, the resultants were incubated in a shaking water bath at 30°C at a rate of 200 rpm for 16 hours. After completion of the incubation, the culture medium was centrifuged to discard the supernatant, the pellet was washed and mixed with a lysis buffer, and the cells were pulverized with a bead homogenizer. The proteins present in the lysate were quantitated according to the Bradford assay, and the activity of isopropylmalate synthase was measured by measuring the CoA produced when the lysate containing proteins (100 μg/mL) was used. The measurement results of the isopropylmalate synthase activity in each strain are shown in Table 3 below.

[Table 3]

Strain	Relative IPMS activity (%)
14067::p117_PCJ7-leuA (WT)	100
14067::p117_PCJ7-leuA (R558H)	105
14067::p117_PCJ7-leuA (G561D)	130
14067::p117_PCJ7-leuA (R558H, G561D)	328

In order to confirm the degree of release of feedback inhibition by leucine in the enzyme, the isopropylmalate synthase activity was measured by measuring the CoA produced when the lysate containing proteins (100 μg/mL) was used under the condition where leucine (3 g/L) was

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added. The measurement results of the isopropylmalate synthase activity in each strain are shown in Table 4 below.

[Table 4]

Strain	Leucine 0 g/L	Leucine 2 g/L
	Relative IPMS activity (%)	
14067::p117_PCJ7-leuA (WT)	100	24
14067::p117_PCJ7-leuA (R558H)	100	61
14067::p117_PCJ7-leuA (G561D)	100	70
14067::p117_PCJ7-leuA (R558H, G561D)	100	89

As shown in Tables 3 and 4 above, it was confirmed that the isopropylmalate synthase activity of the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (R558H), 14067::p117_PCJ7-leuA (G561D), and 14067::p117_PCJ7-leuA (R558H, G561D), which were transformed with the vector expressing the IPMS variant, were enhanced 1.05-fold, 1.3-fold, and 3.2-fold, respectively, compared to that of the control, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (WT). In addition, the L-leucine-producing strains maintained their IPMS activity at 61%, 70%, and 89%, respectively, even when leucine (2 g/L) was added, confirming that feedback inhibition by leucine was released.

Example 9: Production of vector for improving isopropylmalate synthase (IPMS) variant

In Examples 4, 7, and 8, since it was confirmed that the 558th and 561st amino acids in the amino acid sequence (SEQ ID NO: 1) of isopropylmalate synthase were important sites for the activity of the IPMS variant enzyme, the attempt was made to confirm whether the enzyme activity was enhanced or whether feedback inhibition was further released when substituted with an amino acid other than the amino acids in the variant. Therefore, an attempt was made to prepare a variant substituted with an amino acid of other amino acid groups capable of causing structural variations.

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A variant in which the 558th amino acid, arginine, was substituted with alanine (Ala) or glutamine (Gln) was prepared. The vector p117_PCJ7-leuA (R558A), in which the 558th amino acid is substituted with alanine (Ala), and the vector p117_PCJ7-leuA (R558Q), in which the 558th amino acid is substituted with glutamine (Gln), were prepared using a site-directed mutagenesis method and by using the vector p117_PCJ7-leuA (R558H) as a template, the primer of SEQ ID NOs: 13 and 14, and the primer pair of SEQ ID NOs: 15 and 16.

A variant in which the 561st amino acid, glycine, was substituted with arginine (Arg) or tyrosine (Tyr) was prepared. The vector p117_PCJ7-leuA (G561R), in which the 561st amino acid is substituted with arginine (Arg), and the vector p117_PCJ7-leuA (G561Y), in which the 561st amino acid is substituted with tyrosine (Tyr), were obtained using a site-directed mutagenesis method and by using p117_PCJ7-leuA (G561D) as a template, the primer of SEQ ID NOs: 17 and 18, and the primer pair of SEQ ID NOs: 19 and 20.

Example 10: Production of strain in which isopropylmalate-modified variant is introduced

In order to prepare a strain transformed with an expression vector containing the leuA-modified nucleotide sequence prepared in Example 9, wild-type *Corynebacterium glutamicum* ATCC14067 was used as a parent strain.

Each of the vectors, p117_PCJ7-leuA (R558A), p117_PCJ7-leuA (R558Q), p117_PCJ7-leuA (G561R), and p117_PCJ7-leuA (G561Y), which were prepared in Example 9, was transformed in *Corynebacterium glutamicum* ATCC14067 by electroporation to prepare 14067::p117_PCJ7-leuA (R558A), 14067::p117_PCJ7-leuA (R558Q), 14067::p117_PCJ7-leuA (G561R), and 14067::p117_PCJ7-leuA (G561Y).

Example 11: Production of L-leucine in strain in which isopropylmalate synthase-modified variant is introduced

In order to produce L-leucine from the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (R558A), 14067::p117_PCJ7-leuA (R558Q), 14067::p117_PCJ7-leuA (G561R), and 14067::p117_PCJ7-leuA (G561Y), which were prepared

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in Example 10, cultivation was carried out in the following manner.

A platinum loop of each of the parent strain, *Corynebacterium glutamicum* ATCC14067, and the 4 strains above was inoculated into a corner-baffled flask (250 mL) containing a production medium (25 mL). Thereafter, L-leucine was produced by incubating in a shaking water bath at 30°C at a rate of 200 rpm for 60 hours.

After completion of the incubation, the amount of L-leucine produced was measured by high performance liquid chromatography. The concentration of L-leucine in the culture medium for each experimental strain is shown in Table 5 below.

[Table 5] Production of L-leucine in strain overexpressing IPMS variant

Strain	L-Leucine concentration (g/L)
ATCC14067	0.1
14067::p117_PCJ7-leuA (WT)	0.3
14067::p117_PCJ7-leuA (R558H) (Example 7)	4.5
14067::p117_PCJ7-leuA (R558A)	3.8
14067::p117_PCJ7-leuA (R558Q)	3.2
14067::p117_PCJ7-leuA (G561D) (Example 7)	5.1
14067::p117_PCJ7-leuA (G561R)	4.0
14067::p117_PCJ7-leuA (G561Y)	3.6

As shown in Table 5 above, it was confirmed that the L-leucine productivity of the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (R558A) and 14067::p117_PCJ7-leuA (R558Q), was improved 32- to 38-fold compared to the parent strain, *Corynebacterium glutamicum* ATCC14067.

Additionally, it was confirmed that the L-leucine productivity of the L-leucine-producing strains, *Corynebacterium glutamicum* 14067::p117_PCJ7-leuA (G561R) and

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14067::p117_PCJ7-leuA (G561Y), was improved about 36- to 40-fold compared to that of the parent strain, *Corynebacterium glutamicum* ATCC14067.

Based on the results above, it was confirmed the 558th and 561st amino acids in the amino acid sequence (SEQ ID NO: 1) of isopropylmalate synthase were important sites for the activity of the IPMS variant enzyme, and that even when each of the 558th and 561st amino acids of the wild type IPMS protein was substituted with histidine and aspartic acid, respectively, the L-leucine productivity was remarkably increased in the strain having such modification.

While the present disclosure has been described with reference to the particular illustrative embodiments, it will be understood by those skilled in the art to which the present disclosure pertains that the present disclosure may be embodied in other specific forms without departing from the technical spirit or essential characteristics of the present disclosure. Therefore, the embodiments described above are considered to be illustrative in all respects and not restrictive. Furthermore, the scope of the present disclosure is defined by the appended claims rather than the detailed description, and it should be understood that all modifications or variations derived from the meanings and scope of the present disclosure and equivalents thereof are included in the scope of the appended claims.

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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

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Corynebacterium glutamicum* KCJ-24	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11661P
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 January. 22. 2015. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjenae-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: January. 22. 2015.

[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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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

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Corynebacterium glutamicum* KCJ-28	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11662P
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 January, 22, 2015. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjenae-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: January, 22, 2015.

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

INTERNATIONAL FORM

To. CJ CHEILJEDANG
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

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Corynebacterium glutamicum* KCJ-0148	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11811P

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 January. 25. 2016. (date of the original deposit)[1]

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms

Address : Yurim B/D
45, Hongjenae-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: January. 25. 2016.

[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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Claims:

1. A modified microorganism belonging to the genus of *Corynebacterium* for producing L-leucine, comprising a modified polypeptide having an isopropylmalate synthase activity,

wherein glycine at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 is substituted with aspartic acid in the modified polypeptide, and

wherein the modified microorganism has a resistance to norleucine or L-leucine.

2. The modified microorganism of claim 1, wherein a native promoter of a polynucleotide encoding the modified polypeptide is substituted with a promoter which enhances an expression of the polynucleotide compared to the native promoter.

3. The modified microorganism of claim 2, wherein the promoter which enhances the expression of the polynucleotide compared to the native promoter is PCJ7 promoter.

4. The modified microorganism of claim 1, wherein the modified microorganism belonging to the genus of *Corynebacterium* is derived from a *Corynebacterium glutamicum*.

5. A method of producing L-leucine, comprising:

(a) culturing the modified microorganism of any one of claims 1 to 4 in a medium to produce L-leucine; and

(b) recovering L-leucine from the cultured microorganism or the cultured medium.

6. Use of a modified microorganism belonging to the genus of *Corynebacterium* for producing L-leucine, wherein the modified microorganism comprises a modified polypeptide having an isopropylmalate synthase activity,

wherein glycine at position 561 from a N-terminus of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1 is substituted with aspartic acid in the modified polypeptide, and

wherein the modified microorganism has a resistance to norleucine or L-leucine.

7. The use of claim 6, wherein a native promoter of a polynucleotide encoding the modified polypeptide is substituted with a promoter which enhances an

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expression of the polynucleotide compared to the native promoter in the modified microorganism.

8. The use of claim 7, wherein the promoter which enhances the expression of the polynucleotide compared to the native promoter is PCJ7 promoter.

9. The use of claim 6, wherein the modified microorganism belonging to the genus of *Corynebacterium* is derived from a *Corynebacterium glutamicum*.

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