

[DESCRIPTION]

[Invention Title]

MICROORGANISMS FOR PRODUCING PUTRESCINE OR ORNITHINE AND PROCESS FOR PRODUCING PUTRESCINE OR ORNITHINE USING THEM

[Technical Field]

The present invention relates to a recombinant microorganism for producing putrescine or ornithine, and a method for producing putrescine or ornithine using the them.

[Background Art]

Putrescine is found in gram negative bacteria or fungi and present in various species in high concentration. Thus it is expected to carry out important roles in the metabolism of microorganisms. Generally, putrescine is a very important base material for the synthesis of polyamine nylon-4,6 and is mainly produced by a chemical synthesis method. The chemical synthesis method consists of a 3-step process including a catalytic oxidation reaction, a step of using a cyanide compound, and a hydrogenation reaction using high pressure hydrogen. In this regard, for the putrescine production, the development of a more environment-friendly method using biomass which can reduce energy consumption is required.

Under these circumstances, as methods for producing putrescine using a microorganism, methods for high-yield production of putrescine by transformation of *E. coli* and a microorganism of the genus *Corynebacterium* were disclosed (International Patent Publication No. WO 2006/005603; International Patent Publication No. WO 2009/125924; Qian ZD *et al.*, *Biotechnol. Bioeng.* 104 (4): 651 - 662, 2009; Schneider *et al.*, *Appl. Microbiol. Biotechnol.* 88 (4): 859 - 868, 2010; Schneider *et al.*, *Appl. Microbiol. Biotechnol.* 95: 169 - 178, 2012).

Ornithine is a material widely found in plants, animals, and microorganisms and is used as a precursor in the biosynthesis of arginine, proline, and polyamines. Ornithine plays an important role in the pathway for excretion of urea produced from amino acids or ammonia by the ornithine cycle in the *in vivo* metabolism of higher animals. Ornithine is also used as nutrient supplements or pharmaceutical drugs in the industry for improving liver cirrhosis and liver function disorders. The known methods of producing ornithine include treatment of milk

casein with digestive enzymes and use of transformed *E. coli* or a microorganism of the genus *Corynebacterium* (Korean Patent No. 10-1372635; T. Gotoh *et al.*, *Bioprocess Biosyst. Eng.*, 33: 773 - 777, 2010).

SugR, which is a transcriptional regulator of sugar metabolism (hereinafter, SugR), is known as a transcriptional regulator in *Corynebacterium*, and there was a previous report that SugR inhibits the gene encoding PEP-protein phosphotransferase of the PTS system and the genes associated with glycolysis of sugars (VF Wendisch, *et al.*, *J. Bacteriol.* 190: 24, 8033 - 8044, 2008). Citrate synthase is an enzyme that first acts on the TCA cycle and can regulate the rate thereof. There was a report that a modified strain of *Corynebacterium* with reduced GltA activity increased the production of aspartate and lysine (Shiio *et al.*, *Agric Biol Chem.* 46; 101 - 107, 1982).

[Disclosure]

[Technical Problem]

The present inventors have confirmed that the manipulation of *sugR*, the gene encoding SugR, and *gltA*, the gene encoding citrate synthase, improves the putrescine or ornithine productivity, thereby completing the present invention.

[Technical Solution]

An object of the present invention is to provide a recombinant microorganism which can produce putrescine or ornithine in high yield.

Another object of the present invention is to provide a method for producing putrescine or ornithine using the above microorganism.

[Advantageous Effects]

The present inventors have confirmed that simultaneously enhancing the citrate synthase (hereinafter, GltA) activity while weakening the SugR activity in a microorganism of the genus *Corynebacterium* producing putrescine or ornithine increases the amount of putrescine or ornithine production. Accordingly, the microorganism of the present invention can be widely used for the industrial production of putrescine or ornithine, and the microorganism can be widely used as an effective and desirable means in terms of the economical and

environmental aspect to provide base material for the production of various polymer products, in which putrescine or ornithine are used as raw materials.

[Summary]

In one aspect, there is provided a modified microorganism of the genus *Corynebacterium* producing putrescine or ornithine, wherein an activity of transcriptional regulator of sugar metabolism (SugR) is weakened compared to the activity of SugR in the same but unmodified strain of *Corynebacterium* and an activity of citrate synthase (GltA) is enhanced compared to the activity of GltA in the same but unmodified strain of *Corynebacterium*.

In one aspect, there is provided a method for producing putrescine or ornithine, comprising:
(i) culturing the microorganism of the genus *Corynebacterium* as described herein in a medium; and
(ii) recovering putrescine or ornithine from the cultured microorganism or the cultured medium.

[Best Mode for Carrying Out Invention]

An aspect of the present disclosure provides a modified microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of transcriptional regulator of sugar metabolism (SugR) is weakened compared to its endogenous activity, ii) an activity of citrate synthase (GltA) is enhanced compared to its endogenous activity, or iii) the activity of SugR is weakened compared to its endogenous activity and an activity of GltA is enhanced compared to its endogenous activity.

An exemplary embodiment of the present disclosure provides the modified microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of SugR is weakened compared to its endogenous activity and an activity of GltA is enhanced compared to its endogenous activity.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the SugR consists of an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the GltA consists of an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the microorganism of the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium ammoniagenes*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, and *Brevibacterium lactofermentum*.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of ornithine decarboxylase (ODC) is further introduced.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the ODC consists of an amino acid sequence of SEQ ID NO: 17.

Another exemplary embodiment of the present disclosure provides the microorganism of

the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of i) ornithine carbamoyltransferase (ArgF), ii) glutamate exporter, or iii) ornithine carbamoyltransferase and glutamate exporter is further weakened compared to its endogenous activity.

Another exemplary embodiment of the present disclosure further provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the ornithine carbamoyltransferase consists of an amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11, and the glutamate exporter consists of an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 15.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of at least one selected from the group consisting of acetyl-gamma-glutamyl-phosphate reductase (ArgC), acetylglutamate synthase or ornithine acetyltransferase (ArgJ), acetylglutamate kinase (ArgB), and acetylornithine aminotransferase (ArgD) is further enhanced compared to its endogenous activity.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the acetyl-gamma-glutamyl phosphate reductase consists of an amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 21, the acetylglutamate synthase or ornithine acetyltransferase consists of an amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 25, the acetylglutamate kinase consists of an amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29, and the acetylornithine aminotransferase consists of an amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 33.

Another exemplary embodiment of the present disclosure further provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of acetyltransferase is further weakened compared to its endogenous activity.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the acetyltransferase consists of an amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 37.

Another exemplary embodiment of the present disclosure provides the microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of a protein consisting of SEQ ID NO: 39 or SEQ ID NO: 41 is further enhanced compared to its endogenous activity.

Another aspect of the present disclosure provides a method for producing putrescine or

ornithine, including:

(i) culturing the microorganism of the genus *Corynebacterium* producing putrescine or ornithine in a medium; and

(ii) recovering putrescine or ornithine from the cultured microorganism or the cultured medium in step (i).

An exemplary embodiment of the present disclosure provides the method for producing putrescine or ornithine, in which the microorganism of the genus *Corynebacterium* is *Corynebacterium glutamicum*.

Hereinafter, the present disclosure is described in detail.

An aspect of the present disclosure relates to a microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of transcriptional regulator of sugar metabolism (SugR) is weakened compared to its endogenous activity, ii) an activity of citrate synthase (GltA) is enhanced compared to its endogenous activity, or iii) the activity of SugR is weakened compared to its endogenous activity and an activity of GltA is enhanced compared to its endogenous activity. Specifically, the present disclosure relates to a microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which an activity of transcriptional regulator of sugar metabolism is weakened compared to its endogenous activity and an activity of citrate synthase is enhanced compared to its endogenous activity.

As used herein, the term “transcriptional regulator of sugar metabolism (SugR)” refers to an enzyme which broadly functions as an inhibitor regarding the genes associated with various aspects of sugar metabolism, such as sugar uptake and the phosphotransferase system, glycolysis, fermentation related to lactate dehydrogenase, etc. In the present disclosure, SugR includes both the endogenous proteins and foreign proteins within a microorganism of the genus *Corynebacterium*, and specifically, a SugR derived from a microorganism of the genus *Corynebacterium*.

In the present disclosure, the transcriptional regulator of sugar metabolism may include, without limitation, any protein including the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3, or any protein including an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as the

transcriptional regulator of sugar metabolism.

Additionally, since the amino acid sequence of a protein encoding the above activity may differ depending on the species or strain of the microorganism, the SugR may not be limited regarding its origin in the present disclosure, but the SugR may be, for example, derived from a microorganism of the genus *Corynebacterium*, and specifically, derived from *Corynebacterium glutamicum*. It is obvious that any amino acid sequence which has a homology to the above sequences and has a biological activity substantially the same as or corresponding to the protein of SEQ ID NO: 1 or SEQ ID NO: 3 can also belong to the scope of the present disclosure, although the amino acid sequence may have a deletion, modification, substitution, or addition in part of the sequence.

The polynucleotide encoding the transcriptional regulator of sugar metabolism of the present disclosure, as long as it has an activity similar to that of the transcriptional regulator of sugar metabolism, may include any polynucleotide which encodes the protein having an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3, or the polynucleotides which encode proteins having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences. Regarding the polynucleotide encoding the transcriptional regulator of sugar metabolism, considering the codons based on codon degeneracy or those preferred by organisms to express the regulator, various modifications may be executed on the coding region within the scope without changing the amino acid sequence of the polypeptide, and specifically, the polynucleotide may include the polynucleotide sequence of SEQ ID NO: 2 or SEQ ID NO: 4, but is not limited thereto.

As used herein, the term “citrate synthase (GltA)” refers to an enzyme which is involved in the production of various intracellular biosynthetic intermediates and the production of reduced purine nucleic acid. GltA is known to act to mediate the hydrolytic condensation between acetyl-CoA and oxaloacetate for the production of citrate. In the present disclosure, GltA includes both the endogenous enzymes and foreign proteins present in a microorganism of the genus *Corynebacterium*, and specifically, GltA derived from a microorganism of the genus *Corynebacterium*.

In the present disclosure, GltA may include, without limitation, the proteins having the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7, or any protein which includes an

amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, and has the substantial activity of mediating the hydrolytic condensation between acetyl-CoA and oxaloacetate for the production of citrate.

Additionally, since the amino acid sequence of the protein exhibiting the activity may vary according to the species or strain of the microorganism, GltA may be derived from, for example, *Corynebacterium*, and specifically, *Corynebacterium glutamicum*, but the origin of GltA is not limited thereto in the present disclosure. It is obvious that any amino acid sequence which has a homology to the above sequences and has a biological activity substantially the same as or corresponding to the protein of SEQ ID NO: 5 or SEQ ID NO: 7 can also belong to the scope of the present disclosure, although the amino acid sequence may have a deletion, modification, substitution, or addition in part of the sequence.

The polynucleotide encoding GltA of the present disclosure may include the polynucleotides which encode the amino acid of SEQ ID NO: 5 or SEQ ID NO: 7, or the polynucleotides which encode proteins having a homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences. Regarding the polynucleotide encoding GltA, considering the codons based on codon degeneracy or those preferred by organisms to express the GltA, various modifications may be executed on the coding region within the scope without changing the amino acid sequence of the polypeptide, and specifically, the polynucleotide may include the polynucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 8, but is not limited thereto.

As used herein, the term “homology” refers to a degree of identity compared with a given amino acid sequence or a polynucleotide sequence and may be indicated as a percentage. In the present disclosure, homologous sequences having the same or similar activity to that of the given amino acid sequence or the polynucleotide sequence are indicated in terms of “% homology.” For example, homology may be confirmed using standard software for calculating parameters (*e.g.*, parameters such as score, identity, and similarity), specifically BLAST 2.0, or comparing sequences by southern blot under defined stringent hybridization conditions, and the appropriate hybridization conditions to be defined may be determined by a method which is within the scope

of the art and well-known to one of ordinary skill in the art (*e.g.*, J. Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press, Cold Spring Harbor, New York, 1989; F.M. Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York).

Additionally, the polynucleotides encoding SugR and citrate synthase of the present disclosure may be hybridized under stringent conditions with the polynucleotide sequences of SEQ ID NO: 2 or 4, or SEQ ID NO: 6 or 8, or probes derived from the polynucleotide sequences, respectively, and may be a modified type encoding SugR and citrate synthase which are involved in normal functions. As used herein, the term “stringent conditions” refers to a condition which enables a specific hybridization between polynucleotides. For example, the stringent conditions are specifically described in references (*e.g.*, J. Sambrook *et al.*, *ibid*).

In the present disclosure, attempts were made to weaken the activity of SugR, or to enhance the activity of GltA, or to apply both weakening of the SugR activity and enhancing of the GltA activity simultaneously to a microorganism of the genus *Corynebacterium* producing putrescine or ornithine, and as a result, it was confirmed that the amount of putrescine or ornithine production was improved in all modified strains.

In particular, the microorganism of the present disclosure may include both wild type and modified type microorganisms as long as they can produce putrescine or ornithine. For example, the microorganism may belong to the genus *Escherichia*, the genus *Shigella*, the genus *Citrobacter*, the genus *Salmonella*, the genus *Enterobacter*, the genus *Yersinia*, the genus *Klebsiella*, the genus *Erwinia*, the genus *Corynebacterium*, the genus *Brevibacterium*, the genus *Lactobacillus*, the genus *Selenomonas*, the genus *Vibrio*, the genus *Pseudomonas*, the genus *Streptomyces*, the genus *Arcanobacterium*, and the genus *Alcaligenes*. Specifically, the microorganism of the present disclosure may belong to the genus *Corynebacterium*, and more specifically, may be selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium ammoniagenes*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, and *Brevibacterium lactofermentum*, and even more specifically, may be *Corynebacterium glutamicum*, but is not limited thereto.

Specifically, as used herein, the term “producing putrescine or ornithine” refers to a microorganism provided with putrescine or ornithine productivity in a parent strain which has

the putrescine or ornithine in a natural state or has no putrescine or ornithine productivity.

Additionally, the microorganism producing putrescine or ornithine may be modified to weaken an activity of ornithine carbamoyltransferase (ArgF), which is involved in the synthesis of arginine, and/or an activity of glutamate exporter (NCgl1221), which is a protein involved in the excretion of glutamate, compared to their respective endogenous activities.

Furthermore, the microorganism having putrescine productivity may be modified to weaken the activity of acetyltransferase (NCgl1469), which is a protein that acetylates putrescine, compared to its endogenous activity and/or to introduce the activity of ODC, which is a protein that converts ornithine into putrescine.

In particular, the modification of enhancing or weakening activities may occur by a process called transformation in the present disclosure. As used herein, the term “transformation” refers to a process of introducing a polynucleotide encoding a particular protein or a vector including a promoter sequence with strong or weak activity, etc., into a host cell, thereby enabling the expression of the protein encoded by the polynucleotide in the host cell or inducing the modification of the chromosome of the host cell.

Additionally, the polynucleotide includes DNA and RNA which encode the target protein. The polynucleotide may be inserted in any form as long as it can be introduced into a host cell and expressed therein. For example, the polynucleotide may be introduced into a host cell in the form of an expression cassette, which is a gene construct including all essential elements required for self-expression. The expression cassette may conventionally include a promoter operably connected to the polynucleotide, a transcription termination signal, a ribosome-binding domain, and a translation termination signal. The expression cassette may be in the form of an expression vector capable of self-replication. Additionally, the polynucleotide may be introduced into a host cell as it is and operably connected to a sequence necessary for its expression in the host cell, but is not limited thereto.

Additionally, as used herein, the term “operably connected” refers to a functional connection between a promoter sequence, which initiates and mediates the transcription of the polynucleotide encoding the target protein of the present disclosure, and the above gene sequence.

As used herein, the term “vector” refers to any DNA construct which includes the

polynucleotide sequence encoding the target protein, which is operably connected to an appropriate control sequence capable of expressing the target protein in an appropriate host cell. The control sequence includes a promoter capable of initiating transcription, any operator sequence capable of controlling the transcription, a sequence encoding an appropriate mRNA ribosome-binding domain, and sequences capable of controlling the termination of transcription and translation. The vector, after being transformed into an appropriate host cell, may be replicated or function regardless of the host genome, or may be integrated into the host genome itself.

The vector to be used in the present disclosure may not be particularly limited as long as the vector is replicable in a host cell, and any vector known in the art may be used. Examples of the vector conventionally used may include natural or recombinant plasmids, cosmids, viruses, and bacteriophages. For example, as a phage vector or cosmid vector, *pWE15*, *M13*, *MBL3*, *MBL4*, *IXII*, *ASHII*, *APII*, *t10*, *t11*, *Charon4A*, *Charon21A*, etc., may be used; and as a plasmid vector, those based on *pBR*, *pUC*, *pBluescriptII*, *pGEM*, *pTZ*, *pCL*, *pET*, etc., may be used. The vector to be used in the present disclosure may not be particularly limited but any known expression vector may be used. Specifically, *pDZ*, *pDZTn*, *pACYC177*, *pACYC184*, *pCL*, *pECCG117*, *pUC19*, *pBR322*, *pMW118*, *pCC1BAC* vectors, etc., may be used.

As such, the polynucleotide encoding a foreign target protein may be replaced with a modified polynucleotide in the chromosome by a vector for insertion into bacterial chromosome. The insertion of the polynucleotide into the chromosome may be performed using any known method in the art, for example, by homologous recombination, but is not limited thereto. Since the vector of the present disclosure can be inserted into the chromosome by homologous recombination, a selection marker for confirmation of the insertion into the chromosome may be further included. The selection marker is used for the selection of a transformed cell, *i.e.*, to confirm whether the target polynucleotide has been inserted, and markers capable of providing selectable phenotypes such as drug resistance, nutrient requirement, resistance to cytotoxic agents, and expression of surface proteins may be used. Under the circumstances treated with selective agents, only the cells capable of expressing the selection markers can survive or express other phenotypic traits, and thus the transformed cells can be selected.

As used herein, the term “enhancement of activity” not only includes the drawing of a higher effect than the original function due to the new introduction of an activity or an increase

in the activity of a protein itself, but also includes the increase in its activity by an increase in the activity of an endogenous gene, amplification of an endogenous gene from internal or external factor(s), deletion of regulatory factor(s) for inhibiting gene expression, an increase in gene copy number, introduction of a gene from outside, modification of the expression control sequence, and specifically, an increase in enzyme activity due to replacement or modification of a promoter and a mutation within a gene, etc.

Specifically, in the present disclosure, the enhancement or increase of activity may be performed by:

- 1) increasing copy number of a polynucleotide encoding the enzyme,
 - 2) modifying the expression control sequence for increasing the expression of the polynucleotide,
 - 3) modifying the polynucleotide sequence on the chromosome for enhancing the activity of the enzyme, and
 - 4) modifying by a combination thereof,
- but the method is not limited thereto.

The increase of copy number of a polynucleotide (method 1) may be performed in a form in which the polynucleotide is operably linked to a vector, or by inserting the polynucleotide into the chromosome of a host cell, although the method is not particularly limited thereto. Specifically, the increase of copy number of a polynucleotide within the chromosome of the host cell may be performed by introducing a vector which can replicate and function regardless of a host cell and to which the polynucleotide encoding the protein of the present disclosure is operably linked; or may be performed by introducing a vector which can insert the polynucleotide into the chromosome of a host cell and to which the polynucleotide is operably linked, into a host cell.

Then, the modification of the expression control sequence for increasing the expression of a polynucleotide (method 2) may be performed by inducing a modification on the polynucleotide sequence through deletion, insertion, non-conservative or conservative substitution of the polynucleotide sequence, or a combination thereof to further enhance the activity of the expression control sequence, or by replacing the polynucleotide sequence with a polynucleotide sequence having a stronger activity, although the method is not particularly limited thereto. The expression control sequence includes a promoter, an operator sequence, a

sequence encoding ribosome-binding site, and a sequence regulating the termination of transcription and translation.

A strong exogenous promoter, instead of the original promoter, may be connected to the upstream region of the expression unit of the polynucleotide. Examples of the strong promoter may be *CJ7* promoter, *lysCP1* promoter, *EF-Tu* promoter, *groEL* promoter, *aceA* or *aceB* promoter, etc., and more specifically, the expression rate may be improved by being operably connected to *Corynebacterium*-derived *lysCP1* promoter (WO 2009/096689) or *CJ7* promoter (Korean Patent No. 0620092 and WO 2006/065095), but the strong promoter is not limited thereto.

Furthermore, the modification of a polynucleotide sequence on the chromosome (method 3) may be performed by inducing a modification on the expression control sequence through deletion, insertion, non-conservative or conservative substitution of the polynucleotide sequence, or a combination thereof to further enhance the activity of the polynucleotide sequence, or by replacing the polynucleotide sequence with an improved polynucleotide sequence having a stronger activity, although the method is not particularly limited thereto.

As used herein, “weakening of activity” may be achieved by deleting a part or the entirety of a polynucleotide encoding the protein to weaken the activity of the protein, by modifying the expression control sequence to reduce the expression of the polynucleotide, by modifying the polynucleotide sequence on the chromosomes to weaken the activity of the protein, and by a selected method from a combination thereof.

Specifically, in the present disclosure, the weakening of activity may be achieved by:

- 1) deleting a part or the entirety of a polynucleotide encoding the protein,
 - 2) modifying the expression control sequence for reducing the expression of the polynucleotide,
 - 3) modifying the polynucleotide sequence on the chromosomes to weaken the activity of the protein, and
 - 4) a selected method from a combination thereof,
- but the method is not limited thereto.

Specifically, the method of deleting a part or the entirety of a polynucleotide encoding a protein may be performed by replacing the polynucleotide encoding the endogenous target

protein within the chromosome with a polynucleotide having a partial deletion in polynucleotide sequence or a marker gene using a vector for chromosomal insertion within bacteria. As used herein, the term “a part” may vary depending on the kinds of polynucleotides, and it may specifically refer to 1 to 300, more specifically 1 to 100, and even more specifically 1 to 50.

Additionally, the method of modifying the expression control sequence may be performed by inducing a modification on the expression control sequence through deletion, insertion, non-conservative or conservative substitution of a polynucleotide sequence, or a combination thereof to further weaken the activity of the expression control sequence, or by replacing the polynucleotide sequence with a polynucleotide sequence having a weaker activity. The expression control sequence includes a promoter, an operator sequence, a sequence encoding a ribosome-binding site, and a sequence regulating the termination of transcription and translation.

Additionally, the method of modifying a polynucleotide sequence on the chromosome may be performed by inducing a modification on the polynucleotide sequence through deletion, insertion, non-conservative or conservative substitution of the polynucleotide sequence, or a combination thereof to further weaken the activity of the protein, or by replacing the polynucleotide sequence with an improved polynucleotide sequence having a stronger activity.

Additionally, the method of deleting the regulatory factor which inhibits the expression of the polynucleotide of the protein may be performed by replacing the polynucleotide for the expression inhibiting factor with a polynucleotide having a partial deletion in the polynucleotide sequence or a marker gene. As used herein, the term “a part” may vary depending on the kinds of polynucleotides, and it may specifically refer to 1 to 300, more specifically 1 to 100, and even more specifically 1 to 50.

As used herein, the term “endogenous activity” refers to an active state of an enzyme in a non-modified state, *e.g.*, in a natural state, originally possessed by a microorganism, and the term “enhancement compared to its endogenous activity” refers to an increased state of the activity of the protein possessed by the microorganism after manipulation, such as the introduction of a gene exhibiting an activity or an increase of the copy number of the corresponding gene, deletion of the inhibition-control factor of the expression of the gene, or a modification of the expression-control sequence, *e.g.*, the use of an improved promoter, compared to the activity possessed by the microorganism before manipulation.

The microorganism of the genus *Corynebacterium* of the present disclosure may be a microorganism of the genus *Corynebacterium* having putrescine productivity, in which the activity of ornithine decarboxylase (ODC) is further introduced.

As used herein, the term “ornithine decarboxylase (ODC)” refers to an enzyme having putrescine productivity by mediating decarboxylation of ornithine. Although the microorganism of the genus *Corynebacterium* does not have a putrescine biosynthesis pathway, when ODC is introduced from the outside, putrescine is synthesized and released extracellularly. In the present disclosure, the ODC may consist of an amino acid sequence of SEQ ID NO: 17, or may include, without limitation, any protein which has a homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequence, as long as the protein has substantially the same ODC activity.

Additionally, since the amino acid sequence of the protein exhibiting the activity may vary according to the species or strain of the microorganism, the origin of ODC is not limited in the present disclosure, and specifically, it may be an ODC derived from *E. coli*. It is obvious that any amino acid sequence which has a homology to the above sequences and has a biological activity substantially the same as or corresponding to the protein of SEQ ID NO: 17 can also belong to the scope of the present disclosure, although the amino acid sequence may have a deletion, modification, substitution, or addition in part of the sequence.

The polynucleotide encoding ODC of the present disclosure may include the polynucleotides which encode the amino acid of SEQ ID NO: 17, or the polynucleotides which encode proteins having a homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequence. Regarding the polynucleotide encoding ODC, considering the codons based on codon degeneracy or those preferred by organisms to express the protein, various modifications may be executed on the coding region within the scope without changing the amino acid sequence of the polypeptide.

The microorganism of the genus *Corynebacterium* may be a microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which the activities of i) ornithine

carbamoyltransferase (ArgF), ii) glutamate exporter (NCgl1221), or iii) ornithine carbamoyltransferase and glutamate exporter are further weakened compared to their endogenous activities.

In the present disclosure, the ornithine carbamoyltransferase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as ornithine carbamoyltransferase.

Additionally, the glutamate exporter in the present disclosure may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 15, or any protein including an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as the glutamate exporter.

Additionally, the microorganism of the genus *Corynebacterium* of the present disclosure may be a microorganism of the genus *Corynebacterium* producing putrescine or ornithine, in which at least one activity selected from the group consisting of acetyl-gamma-glutamyl phosphate reductase (ArgC), acetylglutamate synthase or ornithine acetyltransferase (ArgJ), acetylglutamate kinase (ArgB), and acetylornithine aminotransferase (ArgD) is further enhanced compared to their endogenous activities.

In the present disclosure, the acetyl-gamma-glutamyl phosphate reductase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 21, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as acetyl-gamma-glutamyl phosphate reductase.

Additionally, the acetylglutamate synthase or ornithine acetyltransferase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 23 or SEQ

ID NO: 25, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as acetylglutamate synthase or ornithine acetyltransferase.

In the present disclosure, the acetylglutamate kinase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as acetylglutamate kinase.

Additionally, in the present disclosure, the acetylornithine aminotransferase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 33, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as acetylornithine aminotransferase.

Furthermore, the microorganism of the genus *Corynebacterium* of the present disclosure may be a microorganism of the genus *Corynebacterium* having putrescine productivity, in which the activity of acetyltransferase (NCgl1469) is further weakened compared to its endogenous activity.

In the present disclosure, the acetyltransferase may include any protein which can transfer an acetyl group to putrescine. The acetyltransferase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 37, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as acetyltransferase.

Lastly, the microorganism of the genus *Corynebacterium* of the present disclosure may be a microorganism of the genus *Corynebacterium* having putrescine productivity, in which the activity of NCgl2522 is further enhanced compared to its endogenous activity.

In the present disclosure, NCgl2522 is a protein playing the role of releasing putrescine, and may include any protein which can transfer an acetyl group to putrescine. The acetyltransferase may include, without limitation, any protein consisting of the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41, or any protein consisting of an amino acid sequence having a sequence homology of 70% or higher, specifically 80% or higher, more specifically 90% or higher, even more specifically 95% or higher, yet even more specifically 98% or higher, and most specifically 99% or higher to the above amino acid sequences, as long as the protein has substantially the same activity as NCgl2522.

In another aspect, the present disclosure provides a method for producing putrescine or ornithine, including:

- (i) culturing the microorganism of the genus *Corynebacterium* producing putrescine or ornithine in a medium; and
- (ii) recovering putrescine or ornithine from the cultured microorganism or the culture in step (i).

In the present disclosure, the microorganism of the genus *Corynebacterium* may be *Corynebacterium glutamicum*.

The microorganism of the genus *Corynebacterium* producing putrescine or ornithine of the present disclosure is the same as described above.

In the above method, the microorganism may be cultured by batch culture, continuous culture, and fed-batch culture known in the art, although they are not particularly limited thereto. In particular, regarding the culturing condition, proper pH (i.e. an optimal pH of 5 to 9, specifically pH 6 to 8, and most specifically pH 6.8) can be maintained using a basic chemical (e.g., sodium hydroxide, potassium hydroxide, or ammonia) or an acidic chemical (e.g., phosphoric acid or sulfuric acid), although it is not particularly limited thereto. Additionally, an aerobic condition can be maintained by adding oxygen or an oxygen-containing gas mixture to a cell culture. The culture temperature may be maintained at 20°C to 45°C, and specifically at 25°C to 40°C, and the microorganism may be cultured for about 10 hours to 160 hours. The

putrescine or ornithine produced by the above culturing may be secreted to a culture medium or remain within the cells.

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

The method of recovering the putrescine or ornithine produced during the culturing of the present disclosure may be performed by an appropriate culture method known in the art, for example, batch culture, continuous culture, or fed-batch culture, and thereby the target material can be recovered from the culture.

[Modes for Carrying Out Invention]

Hereinbelow, the present invention 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 invention.

Example 1: Preparation of *sugR* gene-weakened strains from strains having putrescine productivity

The present inventors have confirmed the effect of weakening of *sugR*, which is the gene encoding SugR, in a strain having putrescine productivity.

1-1. Preparation of *sugR* gene-weakened strains from ATCC13032-based strain

having putrescine productivity

In order to confirm whether the weakening of *sugR* gene is related to the putrescine productivity in a *Corynebacterium glutamicum* ATCC13032-based strain having putrescine productivity (Korean Patent Application Publication No. 10-2013-0082478), a *sugR*-weakened strain was prepared. Specifically, the *sugR*-weakened strain was prepared by changing the initiation codon of the *sugR* gene and replacing the promoter with B6-weakened promoter (Patek M (2005) *Regulation of gene expression*. In: Eggeling L, Bott M (eds) *Handbook of Corynebacterium glutamicum*. CRC, BocaRaton).

First, a vector for changing the initiation codon of the *sugR* gene was prepared. Regarding the vicinity of the polynucleotide sequence of the gene encoding the SugR described by SEQ ID NO: 2, the primer pairs of SEQ ID NOS: 43 and 44 for obtaining the homologous recombinant fragment upstream of the initiation codon of the *sugR* gene and the primer pairs of SEQ ID NOS: 45, 46 and 47 for obtaining the homologous recombinant fragment downstream of the initiation codon of the *sugR* gene were prepared. The primers used for the change of the initiation codons are summarized in Table 1 below.

[Table 1]

Primer	Sequence (5' -> 3')
sugR F1_ SalI (SEQ ID NO: 43)	CTTGCATGCCTGCAGGTCGACAGGATTCATCTG GCATCTGGC
sugR -R1 (SEQ ID NO: 44)	GTCACTCCTTAAAGCAAAAAGCC
sugR -F2_GTG (SEQ ID NO: 45)	TTTTTGCTTTAAGGAGTGACGTGTACGCAGAGG AGCGCCGTC
sugR -F2_TTG (SEQ ID NO: 46)	TTTTTGCTTTAAGGAGTGACTTGTACGCAGAGG AGCGCCGTC
sugR -R2_BamHI (SEQ ID NO: 47)	CGAGCTCGGTACCCGGGGATCCGCGAGAGTACG AAGCGCAGT

PCR was performed using the genomic DNA of *Corynebacterium glutamicum* ATCC13032 as a template along with 2 pairs of primers, respectively, to amplify the upstream region and the downstream region of the initiation codon of *sugR* gene, respectively, and the

resultants were subjected to electrophoresis to obtain desired fragments. In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. The thus-obtained fragments were subjected to electrophoresis in a 0.8% agarose gel, and the bands of desired sizes were eluted and purified.

The *pDZ* vector (Korean Patent No. 10-0924065) was treated with *Bam*HI and *Sal*I and then the PCR products of the *ATCC13032* strain were subjected to fusion cloning. The fusion cloning was performed using the In-Fusion® HD Cloning Kit (Clontech). As such, plasmids *pDZ-1'sugR(GTG)* and *pDZ-1'sugR(TTG)* were prepared.

In the case of the vector for the replacement into a B6-weakened promoter, SEQ ID NO: 48 for the vector preparation was prepared as shown in Table 2 below.

[Table 2]

Primer	Sequence (5' -> 3')
sugR F3 (SEQ ID NO: 48)	TTTTTGCTTTAAGGAGTGACGAAGGCAACCATGAAC TCTAATGTACGCAGAGGAGCGCCGTC

PCR was performed using the primer pairs of SEQ ID NOS: 43 and 44 for obtaining the homologous recombinant fragment upstream of the initiation codon of the *sugR* gene and the primer pairs of SEQ ID NOS: 48 and 47 for obtaining the homologous recombinant fragment downstream of the initiation codon of the *sugR* gene, which were prepared regarding the vicinity of the polynucleotide sequence of the gene encoding the SugR described by SEQ ID NO: 2, and the upstream region and the downstream region of the initiation codon of *sugR* gene were amplified, respectively, and the resultants were subjected to electrophoresis to obtain desired fragments. The thus-obtained fragments were subjected to electrophoresis in a 0.8% agarose gel, and the bands of desired sizes were eluted and purified. The *pDZ* vector was treated with *Bam*HI and *Sal*I and then the PCR products of the *ATCC13032* strain were subjected to fusion cloning. The fusion cloning was performed using the In-Fusion® HD Cloning Kit (Clontech). As such, the plasmid *pDZ-1'sugR(B6)* was prepared.

The plasmids *pDZ-1'sugR(GTG)*, *pDZ-1'sugR(TTG)*, and *pDZ-1'sugR(B6)* were introduced into a microorganism of the genus *Corynebacterium* KCCM11240P (Korean Patent Application Publication No. 10-2013-0082478) by electroporation to obtain transformants, and the transformants were plated on BHIS plate media (Braine heart infusion 37 g/L, sorbitol 91 g/L,

and agar 2%) containing kanamycin (25 µg/mL) and X-gal (5-bromo-4-chloro-3-indolin-*D*-galactoside) and cultured to obtain colonies. Among the colonies, blue colonies were selected and thereby the strains introduced with the plasmids *pDZ-1'sugR(GTG)*, *pDZ-1'sugR(TTG)*, and *pDZ-1'sugR(B6)* were selected.

The selected strains were cultured with shaking (30°C, 8 hours) in CM medium (glucose 10 g/L, polypeptone 10 g/L, yeast extract 5 g/L, beef extract 5 g/L, NaCl 2.5 g/L, urea 2 g/L, pH 6.8) and sequentially diluted from 10⁻⁴ to 10⁻¹⁰, plated on a solid medium containing X-gal, and cultured to form colonies.

Among the thus-formed colonies, white colonies which appeared at a relatively low rate were selected and the strains in which the initiation codon of *sugR* was changed into GTG or TTG by a secondary crossover or the strains in which the promoter was changed into B6 were finally selected. Regarding the finally selected strains, PCR was performed using a primer pair of SEQ ID NOS: 43 and 47 and confirmed that the initiation codon of *sugR* was changed into GTG or TTG, or the promoter was converted to a B6-weakened promoter, and the modified strains of *Corynebacterium glutamicum* were named as KCCM11240P *sugR* (GTG), KCCM11240P *sugR* (TTG), KCCM11240P *sugR* (B6).

1-2. Preparation of a *sugR* gene-weakened strain from an ATCC13869-based strain having putrescine productivity

DAB12-a ΔNCgl1469 (Korean Patent Application Publication No. 10-2014-0115244), which is a *Corynebacterium glutamicum* ATCC13869-based strain having putrescine productivity, was named as DAB12-b, and a *sugR*-weakened strain was prepared based on the DAB12-b strain.

Specifically, in order to confirm the sequences of the gene encoding SugR derived from *Corynebacterium glutamicum* ATCC13869 and the protein expressed therefrom, PCR was performed using the genomic DNA of *Corynebacterium glutamicum* ATCC13869 as a template along with a primer pair of SEQ ID NO: 43 and SEQ ID NO: 49.

[Table 3]

Primer	Sequence (5' -> 3')
<i>sugR</i> R (SEQ ID NO: 49)	GGACTTGCAGTGACTGTAAGAA

In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute and 30 seconds.

The thus-obtained PCR products were separated by electrophoresis and subjected to sequence analysis, and as a result, it was confirmed that the gene encoding SugR derived from *Corynebacterium glutamicum* ATCC13869 includes a polynucleotide sequence described by SEQ ID NO: 4. The comparison of the protein sequence being encoded therefrom and the amino acid sequence of the SugR derived from *Corynebacterium glutamicum* ATCC13032 (SEQ ID NO: 1) revealed that their homology was 99%.

In order to change the initiation codon of *sugR* derived from *Corynebacterium glutamicum* ATCC13869 and replace the B6-weakened promoter, PCR was performed as in Example 1-1 using the genomic DNA of *Corynebacterium glutamicum* ATCC13869 as a template along with the primers described in Tables 1 and 2 above, and the PCR fragments of the upstream region and the downstream region of the initiation codon of *sugR* were amplified, respectively, and then subjected to electrophoresis to obtain the desired fragments. In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. The thus-obtained fragments were subjected to electrophoresis in a 0.8% agarose gel, and the bands of desired sizes were eluted and purified.

The *pDZ* vector was treated with *Bam*HI and *Sal*I and then the PCR products of the ATCC13032 strain were subjected to fusion cloning. The fusion cloning was performed using the In-Fusion® HD Cloning Kit (Clontech). As such, the plasmids *pDZ-2'sugR(GTG)*, *pDZ-2'sugR(TTG)*, and *pDZ-2'sugR(B6)* were prepared.

The plasmids *pDZ-2'sugR(GTG)*, *pDZ-2'sugR(TTG)*, and *pDZ-2'sugR(B6)* were transformed into *Corynebacterium glutamicum* DAB12-b in the same manner as in Example 1-1, and the strains in which the initiation codon of *sugR* was changed and/or the promoter was converted to a B6-weakened promoter were selected. The thus-selected modified strains of *Corynebacterium glutamicum* were named as DAB12-b *sugR(GTG)*, DAB12-b *sugR(TTG)*, and DAB12-b *sugR(B6)*, respectively.

Example 2: Preparation of a *gltA*-enhanced strain from a strain having putrescine productivity

In order to confirm the effect of enhancing the activity of *gltA*, which is citrate synthase, in a strain having putrescine productivity, a modified strain was prepared in which *gltA* gene was introduced in a transposon gene within the chromosome of the strain having putrescine productivity. The *pDZTn* vector (WO 2009/125992) for transformation, which can introduce a gene into the chromosome, and the region of the transposon gene of the microorganism of the genus *Corynebacterium* was used.

2-1. Preparation of a *gltA*-enhanced strain from an ATCC13032-based strain having putrescine productivity

The fragments of *gltA* gene were amplified using the genomic DNA of *Corynebacterium glutamicum* ATCC13032 as a template along with the primers of SEQ ID NOS: 50 and 51 (Table 4). In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds or 1 minute and 30 seconds. The thus-obtained fragments were subjected to electrophoresis in a 0.8% agarose gel, and the bands of desired sizes were eluted and purified.

The *pDZTn* vector was treated with *SpeI* and then the PCR products were subjected to fusion cloning, respectively. The fusion cloning was performed using the In-Fusion® HD Cloning Kit (Clontech). The thus-obtained plasmid was named as *pDZTnI'-gltA*.

[Table 4]

Primer	Sequence (5' -> 3')
<i>gltA</i> F_ <i>speI</i> (SEQ ID NO: 50)	GAAGGAATGAGTTCCTCGAGACTAGTACTCGGCACCCA TCCTTGTC
<i>gltA</i> R_ <i>speI</i> (SEQ ID NO: 51)	GTTATTAGATGTCTGGGCCCCACTAGTGTGCTGTACATGC TCCTTGAAAATC

The thus-prepared plasmid was introduced into the KCCM11240P strain by electroporation to obtain a transformant, and the transformant was cultured with shaking (30°C, 8 hours) in CM medium (glucose 10 g/L, polypeptone 10 g/L, yeast extract 5 g/L, beef extract 5 g/L, NaCl 2.5 g/L, urea 2 g/L, pH 6.8), sequentially diluted from 10⁻⁴ to 10⁻¹⁰, plated on a solid medium containing X-gal, and cultured to form colonies.

Among the thus-formed colonies, white colonies which appeared at a relatively low rate were selected and the strain in which the gene encoding *gltA* was introduced by a secondary

crossover was finally selected. Regarding the finally selected strain, it was confirmed that PCR was performed using a primer pair of SEQ ID NOS: 50 and 51 and that the gene encoding *gltA* was introduced therein, and the modified strain of *Corynebacterium glutamicum* was named as KCCM11240P Tn::*gltA*.

2-2. Preparation of a *gltA*-enhanced strain from an ATCC13869-based strain having putrescine productivity

Regarding the *DAB12-b* strain used in Example 1-2, a *gltA*-enhanced strain was prepared.

Specifically, in order to confirm the sequences of the gene encoding *gltA* derived from *Corynebacterium glutamicum* ATCC13869 and the protein expressed therefrom, PCR was performed using the genomic DNA of *Corynebacterium glutamicum* ATCC13869 as a template along with a primer pair of SEQ ID NO: 50 and SEQ ID NO: 51.

In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute and 30 seconds. The thus-obtained PCR products were separated by electrophoresis and subjected to sequence analysis, and as a result, it was confirmed that the gene encoding *gltA* derived from *Corynebacterium glutamicum* ATCC13869 includes a polynucleotide sequence described by SEQ ID NO: 8. The comparison of the protein sequence being encoded therefrom and the amino acid sequence of the *gltA* derived from *Corynebacterium glutamicum* ATCC13032 (SEQ ID NO: 5) revealed that their homology was 99%.

In order to enhance the *gltA* derived from *Corynebacterium glutamicum* ATCC13869, PCR was performed as in Example 2-1 using the genomic DNA of *Corynebacterium glutamicum* ATCC13869 as a template along with the primers of SEQ ID NOS: 50 and 51 to amplify fragments of the gene. In particular, PCR was performed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds or 1 minute and 30 seconds. The thus-obtained PCR fragments were subjected to electrophoresis in a 0.8% agarose gel, and the bands of desired sizes were eluted and purified.

The *pDZTn* vector was treated with *SpeI* and then the PCR products were subjected fusion cloning, respectively. The fusion cloning was performed using the In-Fusion® HD Cloning Kit (Clontech). The thus-obtained plasmid was named as *pDZTn2'-gltA*. The plasmid *pDZTn2'-gltA* was transformed into the *Corynebacterium glutamicum* *DAB12-b* strain

in the same manner as in Example 2-1 and thereby the strain in which *gltA* was enhanced was selected. The thus-selected modified strain of *Corynebacterium glutamicum* was named as *DAB12-b Tn:gltA*.

Example 3: Preparation of strains having putrescine productivity with the integration of *sugR*-weakening and *gltA*-enhancement and confirmation of the putrescine productivity of the strains

In order to confirm the improvement of putrescine productivity of the *sugR*-weakened strains prepared in Examples 1-1 and 1-2 by the insertion of the *gltA* gene, the *gltA* gene was introduced into the transposon gene. In particular, the vectors *pDZTn1'-gltA* and *pDZTn2'-gltA* prepared in Examples 2-1 and 2-2 were used.

Specifically, the plasmid *pDZTn1'-gltA* was transformed into *Corynebacterium glutamicum* KCCM11240P *sugR*(GTG), -KCCM11240P *sugR*(TTG), and -KCCM11240P *sugR*(B6) in the same manner as in Example 2-1 to prepare *gltA*-enhanced strains. The thus-prepared modified strains of *Corynebacterium glutamicum* were named as KCCM11240P *sugR*(GTG) *Tn::gltA*, KCCM11240P *sugR*(TTG) *Tn::gltA*, and KCCM11240P *sugR*(B6) *Tn::gltA*, respectively, and among them, KCCM11240P *sugR*(TTG) *Tn::gltA* (*Corynebacterium glutamicum* CC01-1147) was deposited with the Korean Culture Center of Microorganisms (KCCM) on November 28, 2014, under the accession number KCCM11615P.

Additionally, the plasmid *pDZTn2'-gltA* was transformed into *Corynebacterium glutamicum* *DAB12-b sugR*(GTG), -*DAB12-b sugR*(TTG), and -*DAB12-b sugR*(B6) in the same manner as in Example 2-2 to prepare *gltA*-enhanced strains. The thus-prepared modified strains of *Corynebacterium glutamicum* were named as *DAB12-b sugR*(GTG) *Tn::gltA*, *DAB12-b sugR*(TTG) *Tn::gltA*, and *DAB12-b sugR*(B6) *Tn::gltA*, respectively.

Example 4: Evaluation of putrescine productivity of strains having putrescine productivity with the integration of *sugR*-weakening and *gltA*-enhancement

In order to confirm the effect of *sugR*-weakening and *gltA*-enhancement in strains having putrescine productivity on the production of putrescine, the putrescine productivity was compared among the modified strains of *Corynebacterium glutamicum* having putrescine productivity prepared in Examples 1, 2, and 3.

Specifically, 6 different kinds of modified strains of *Corynebacterium glutamicum*, i.e., (KCCM11240P sugR (GTG) Tn::gltA/ KCCM11240P sugR (TTG) Tn::gltA/ KCCM11240P sugR (B6) Tn::gltA/ DAB12-b sugR (GTG) Tn::gltA/ DAB12-b sugR (TTG) Tn::gltA, and DAB12-b sugR (B6) Tn::gltA)), and 2 different kinds of parent strains (i.e., KCCM11240P and DAB12-b) were respectively plated on 1 mM arginine-containing CM plate media (1% glucose, 1% polypeptone, 0.5% yeast extract, 0.5% beef extract, 0.25% NaCl, 0.2% urea, 100 µl of 50% NaOH, 2% agar, pH 6.8, based on 1 L), and cultured at 30°C for 24 hours.

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% (NH₄)₂SO₄, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% CaCO₃, based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours.

In all cultures of strains, 1 mM arginine was added to the media. Upon completion of culture, the concentration of putrescine produced in each culture was measured and the results are shown in Table 5 below.

[Table 5]

Strain	Putrescine (g/L)	Productivity (g/L/h)	Fold (%)
KCCM11240P	5.8	0.116	100
KCCM11240P sugR (TTG)	6.3	0.126	109
KCCM11240P sugR (GTG)	6.3	0.126	109
KCCM11240P sugR (B6)	6.0	0.120	103
KCCM11240P Tn::gltA	6.2	0.124	107
KCCM11240P sugR (TTG) Tn::gltA	6.8	0.136	117
KCCM11240P sugR (GTG) Tn::gltA	6.5	0.130	112
KCCM11240P sugR (B6) Tn::gltA	6.3	0.126	109
DAB12-b	6.5	0.129	100
DAB12-b sugR (TTG)	6.9	0.138	107
DAB12-b sugR (GTG)	6.8	0.136	105
DAB12-b sugR (B6)	6.7	0.134	104

DAB12-b Tn::gltA	7.0	0.140	109
DAB12-b sugR (TTG) Tn::gltA	7.3	0.146	113
DAB12-b sugR (GTG) Tn::gltA	7.1	0.142	110
DAB12-b sugR (B6) Tn::gltA	7.1	0.142	110

As shown in Table 5 above, the modified strains of *Corynebacterium glutamicum* with weakened sugR or enhanced gltA showed an increase in the putrescine productivity compared to the non-modified strain, KCCM11240P, by 3% to 9%, and also, the modified strains of *Corynebacterium glutamicum* with simultaneously weakened sugR and enhanced gltA showed an increase in the putrescine productivity by 9% to 17%.

Additionally, the modified strains of the DAB12-b strain with weakened sugR or enhanced gltA showed an increase in the putrescine productivity compared to the non-modified strain, by 4% to 9% and also, the modified strains of the DAB12-b strain with simultaneously weakened sugR and enhanced gltA showed an increase in the putrescine productivity by 10% to 13%.

Example 5: Preparation of strains with increased ability of putrescine secretion based on strains having putrescine productivity with integration of sugR-weakening and gltA-enhancement and confirmation of putrescine productivity of the strains

5-1. Preparation of strains with increased ability of putrescine secretion based on strains with integration of sugR-weakening and gltA-enhancement

In order to confirm whether the KCCM11401P strain with increased ability of putrescine secretion (Korean Patent Application Publication No. 10-2014-0115244) can improve the putrescine productivity by the weakening of the activity of *sugR* gene and the enhancement of the activity of *gltA* gene, modified strains were prepared.

Specifically, first, the plasmids *pDZ-1'sugR(GTG)*, *pDZ-1'sugR(TTG)*, and *pDZ-1'sugR(B6)* prepared in Example 1-1 were transformed into *Corynebacterium glutamicum* KCCM 11401P, and the strains in which the initiation codon of *sugR* was converted to TTG thus resulted in weakening of *sugR* were selected therefrom. The thus-selected modified strains of *Corynebacterium glutamicum* were named as KCCM11401P *sugR*(GTG), KCCM11401P *sugR*(TTG), and KCCM11401P *sugR*(B6), respectively.

Then, in order to confirm whether the putrescine productivity can be improved by the enhancement of the activity of *gltA* gene, the *gltA* gene was introduced into a transposon gene of the strains with weakened *sugR* gene prepared above. In particular, the vector *pDZTn1'-gltA* prepared in Example 2-1 was used.

Specifically, the plasmid *pDZTn1'-gltA* prepared in Example 2-1 was transformed into KCCM11401P *sugR*(GTG), KCCM11401P *sugR*(TTG), and KCCM11401P *sugR*(B6), and the *gltA*-enhanced strains were selected. The thus-selected modified strains of *Corynebacterium glutamicum* were named as KCCM11401P *sugR*(GTG) Tn::*gltA*, KCCM11401P *sugR*(TTG) Tn::*gltA*, and KCCM11401P *sugR*(B6) Tn::*gltA*.

5-2. Evaluation of strains with increased ability of putrescine secretion based on strains having putrescine productivity with integration of *sugR*-weakening and *gltA*-enhancement regarding putrescine productivity

In order to confirm the effect of *sugR*-weakening and *gltA*-enhancement in strains having putrescine productivity regarding their production of putrescine, the putrescine productivity was compared among the modified strains of *Corynebacterium glutamicum* prepared in Example 5-1.

Specifically, 7 different kinds of modified strains of *Corynebacterium glutamicum* (i.e., (KCCM11401P *sugR*(GTG), KCCM11401P *sugR*(TTG), KCCM11401P *sugR*(B6), KCCM11401P Tn::*gltA*, KCCM11401P *sugR*(GTG) Tn::*gltA*, KCCM11401P *sugR*(TTG) Tn::*gltA*, and KCCM11401P *sugR*(B6) Tn::*gltA*) and a single parent strain (KCCM11401P) were respectively plated on 1 mM arginine-containing CM plate media (1% glucose, 1% polypeptone, 0.5% yeast extract, 0.5% beef extract, 0.25% NaCl, 0.2% urea, 100 µl of 50% NaOH, 2% agar, pH 6.8, based on 1 L), and cultured at 30°C for 24 hours.

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% (NH₄)₂SO₄, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% CaCO₃ 1 L, based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours.

[Table 6]

Strain	Putrescine (g/L)	Productivity	Fold
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		(g/L/h)	(%)
KCCM11401P	5.3	0.106	100
KCCM11401P sugR (TTG)	5.6	0.112	106
KCCM11401P sugR (GTG)	5.5	0.110	104
KCCM11401P sugR (B6)	5.4	0.108	102
KCCM11401P Tn::gltA	5.6	0.112	106
KCCM11401P sugR (TTG) Tn::gltA	6.1	0.122	115
KCCM11401P sugR (GTG) Tn::gltA	5.9	0.118	111
KCCM11401P sugR (B6) Tn::gltA	5.8	0.116	109

As shown in Table 6 above, the modified strains of *Corynebacterium glutamicum* with weakened sugR or enhanced gltA showed an increase in the putrescine productivity compared to the non-modified strain, KCCM11401P, by 2% to 6%, and also, the modified strains of *Corynebacterium glutamicum* with simultaneously weakened sugR and enhanced gltA showed an increase in the putrescine productivity by 9% to 15% in the putrescine productivity. It was confirmed that the results agreed with the interpretation of the results of Table 5.

Example 6. Preparation of sugR-weakened strains from a strain having ornithine productivity

In order to confirm whether the weakening of sugR derived from *Corynebacterium glutamicum* ATCC13032 has an effect on the ornithine productivity, modified strains were prepared using the vectors prepared in Example 1-1.

The plasmids prepared in Example 1-1, i.e., pDZ-1'sugR(GTG), pDZ-1'sugR(TTG), and pDZ-1'sugR(B6), were introduced into the KCCM11137P strain (Korean Patent No. 10-1372635), which was prepared using *Corynebacterium glutamicum* ATCC13032 as the parent strain, by electroporation to obtain transformants, and the transformants were plated on BHIS plate media (Braine heart infusion 37 g/L, sorbitol 91 g/L, and agar 2%) containing kanamycin (25 µg/mL) and X-gal (5-bromo-4-chloro-3-indolin-D-galactoside) and cultured to obtain colonies. Among the colonies, blue colonies were selected and thereby the strains introduced with the plasmids pDZ-1'sugR(GTG), pDZ-1'sugR(TTG), and pDZ-1'sugR(B6) were selected.

The selected strains were cultured with shaking (30°C, 8hours) in CM medium (glucose 10 g/L, polypeptone 10 g/L, yeast extract 5 g/L, beef extract 5 g/L, NaCl 2.5 g/L, urea 2 g/L, pH

6.8) and sequentially diluted from 10^{-4} to 10^{-10} , plated on a solid medium containing X-gal, and cultured to form colonies. Among the thus-formed colonies, white colonies which appeared at a relatively low rate were selected and the strains, in which the initiation codon of *sugR* was changed into GTG or TTG by a secondary crossover or the strains in which the promoter was changed into B6 were finally selected. Regarding the finally selected strains, PCR was performed using a primer pair of SEQ ID NO: 43 and 47 and then it confirmed that the initiation codon of *sugR* was changed into GTG or TTG. The obtained modified strains of *Corynebacterium glutamicum* were named as KCCM11137P *sugR*(GTG), KCCM11137P *sugR*(TTG), and KCCM11137P *sugR*(B6), respectively.

Example 7. Preparation of *gltA*-enhanced strains from strains having ornithine productivity

In order to confirm the effect of enhancing the *gltA* gene in a strain having ornithine productivity on its production of ornithine, a modified strain was prepared by inserting the *gltA* gene into the chromosome of the strain having ornithine productivity using the vectors prepared in Example 2-1.

Specifically, the vectors prepared in Example 2-1 were introduced into the KCCM11137P strain (Korean Patent No. 10-1372635) by electroporation to obtain transformants, and the transformants were cultured with shaking (30°C, 8 hours) in CM medium (glucose 10 g/L, polypeptone 10 g/L, yeast extract 5 g/L, beef extract 5 g/L, NaCl 2.5 g/L, urea 2 g/L, pH 6.8), sequentially diluted from 10^{-4} to 10^{-10} , plated on a solid medium containing X-gal, and cultured to form colonies.

Among the thus-formed colonies, white colonies which appeared at a relatively low rate were selected and the strain in which the gene encoding *gltA* was introduced by a secondary crossover was finally selected. Regarding the finally selected strain, PCR was performed using a primer pair of SEQ ID NOS: 50 and 51 and confirmed that the gene encoding *gltA* was introduced therein, and the modified strain of *Corynebacterium glutamicum* was named as KCCM11137P Tn::*gltA*.

Example 8. Preparation of strains with the integration of *sugR*-weakening and *gltA*-enhancement and confirmation of the putrescine productivity of the strains

8-1. Preparation of ATCC13032-based strains having ornithine productivity with the integration of sugR-weakening and gltA-enhancement

In order to confirm the effect of enhancing the activity of ornithine productivity in sugR-weakened KCCM11137P sugR(GTG), KCCM11137P sugR(TTG), and KCCM11137P sugR(B6) prepared in Example 6 by the insertion of the *gltA* gene into the chromosome, the *gltA* gene was introduced into a transposon gene. In particular, the vector *pDZTn1'-gltA* prepared in Example 2-1 was used.

The plasmid *pDZTn1'-gltA* was transformed into the *Corynebacterium glutamicum* KCCM11137P sugR TTG) in the same manner as in Example 2-1 and *gltA*-enhanced strains were selected. The thus-selected modified strains of *Corynebacterium glutamicum* were named as KCCM11137P sugR(GTG) Tn::*gltA*, KCCM11137P sugR(TTG) Tn::*gltA*, and KCCM11137P sugR(B6) Tn::*gltA*, respectively.

8-2. Evaluation of strains with integration of sugR-weakening and gltA-enhancement on ornithine productivity

In order to confirm the effect of sugR-weakening and *gltA*-enhancement in strains having ornithine productivity regarding their production of ornithine, the ornithine productivity was compared among the modified strains of *Corynebacterium glutamicum* prepared in Example 8-1.

Specifically, 7 different kinds of modified strains of *Corynebacterium glutamicum* (i.e., (KCCM11137P sugR(GTG), KCCM11137P sugR(TTG), KCCM11137P sugR(B6), KCCM11137P Tn::*gltA*, KCCM11137P sugR(GTG) Tn::*gltA*, KCCM11137P sugR(TTG) Tn::*gltA*, and KCCM11137P sugR(B6) Tn::*gltA*) and a single parent strain (KCCM11137P) were respectively plated on 1 mM arginine-containing CM plate media (1% glucose, 1% polypeptone, 0.5% yeast extract, 0.5% beef extract, 0.25% NaCl, 0.2% urea, 100 µl of 50% NaOH, 2% agar, pH 6.8, based on 1 L), and cultured at 30°C for 24 hours.

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% (NH₄)₂SO₄, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% CaCO₃ based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours. In all cultures of strains, 1 mM

arginine was added to the media. Upon completion of culture, the concentration of ornithine produced in each culture was measured and the results are shown in Table 7 below.

[Table 7]

Strain	Ornithine (g/L)	Productivity (g/L/h)	Fold (%)
KCCM11137P	11.5	0.230	100
KCCM11137P sugR(TTG)	12.5	0.250	109
KCCM11137P sugR(GTG)	12.3	0.246	107
KCCM11137P sugR(B6)	12.5	0.250	109
KCCM11137P Tn::gltA	12.4	0.248	108
KCCM11137P sugR(TTG) Tn::gltA	13.5	0.270	117
KCCM11137P sugR(GTG) Tn::gltA	13	0.260	113
KCCM11137P sugR(B6) Tn::gltA	12.9	0.258	112

As shown in Table 7 above, the modified strains of *Corynebacterium glutamicum* with weakened sugR or enhanced gltA showed an increase in the ornithine productivity compared to the non-modified strain, KCCM11137P, by 7% to 9%, and also, the modified strains of *Corynebacterium glutamicum* with simultaneously weakened sugR and enhanced gltA showed an increase in the ornithine productivity by 12% to 17% in the ornithine productivity.

Conclusively, in a *Corynebacterium* strain producing putrescine or ornithine, it was confirmed that the production of putrescine and ornithine can be increased by weakening sugR or enhancing gltA, and when gltA was enhanced while weakening sugR, the production of putrescine and ornithine was increased more significantly.

From the foregoing, a skilled person in the art to which the present invention pertains will be able to understand that the present invention may be embodied in other specific forms without modifying the technical concepts or essential characteristics of the present invention. In this regard, the exemplary embodiments disclosed herein are only for illustrative purposes and should not be construed as limiting the scope of the present invention. On the contrary, the present invention is intended to cover not only the exemplary embodiments but also various alternatives, modifications, equivalents, and other embodiments that may be included within the spirit and

scope of the present invention as defined by the appended claims.



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 : <i>Corynebacterium glutamicum</i> CC01-1147	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11615P
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 November, 28, 2014. (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjense-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: November, 28, 2014.



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.

Form BP/4

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CLAIMS

1. A modified microorganism of the genus *Corynebacterium* producing putrescine or ornithine, wherein an activity of transcriptional regulator of sugar metabolism (SugR) is weakened compared to the activity of SugR in the same but unmodified strain of *Corynebacterium* and an activity of citrate synthase (GltA) is enhanced compared to the activity of GltA in the same but unmodified strain of *Corynebacterium*.
2. The microorganism according to claim 1, wherein the transcriptional regulator of sugar metabolism consists of an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3.
3. The microorganism according to claim 1, wherein the citrate synthase consists of an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7.
4. The microorganism according to claim 1, wherein the microorganism of the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium ammoniagenes*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, and *Brevibacterium lactofermentum*.
5. The microorganism according to claim 1, wherein an activity of ornithine decarboxylase (ODC) is further introduced.
6. The microorganism according to claim 5, wherein the ornithine decarboxylase consists of an amino acid sequence of SEQ ID NO: 17.
7. The microorganism according to claim 1, wherein an activity of
 - i) ornithine carbamoyltransferase (ArgF) is further weakened compared to the activity of ArgF in the same but unmodified strain of *Corynebacterium*,
 - ii) a glutamate exporter is further weakened compared to the activity of the glutamate exporter in the same but unmodified strain of *Corynebacterium*, or
 - iii) ornithine carbamoyltransferase and a glutamate exporter is further weakened compared to the activity of ornithine carbamoyltransferase and the glutamate exporter in the same but unmodified strain of *Corynebacterium*.
8. The microorganism according to claim 7, wherein the ornithine carbamoyltransferase consists of an amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11, and the glutamate exporter consists of an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 15.
9. The microorganism according to claim 1, wherein an activity of at least one enzyme

selected from the group consisting of acetyl-gamma-glutamyl-phosphate reductase (ArgC), acetylglutamate synthase or ornithine acetyltransferase (ArgJ), acetylglutamate kinase (ArgB), and acetylornithine aminotransferase (ArgD) is further enhanced compared to the activity of said enzyme(s) in the same but unmodified strain of *Corynebacterium*.

10. The microorganism according to claim 9, wherein the acetyl-gamma-glutamyl-phosphate reductase consists of an amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 21, the acetylglutamate synthase or ornithine acetyltransferase consists of an amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 25, the acetylglutamate kinase consists of an amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29, and the acetylornithine aminotransferase consists of an amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 33.

11. The microorganism according to claim 1, wherein an activity of acetyltransferase is further weakened compared to the activity of acetyltransferase in the same but unmodified strain of *Corynebacterium*.

12. The microorganism according to claim 11, wherein the acetyltransferase consists of an amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 37.

13. The microorganism of claim 1, wherein an activity of a protein consisting of SEQ ID NO: 39 or SEQ ID NO: 41 is further enhanced compared to the same activity of said protein in the same but unmodified strain of *Corynebacterium*.

14. A method for producing putrescine or ornithine, comprising:

- (i) culturing the microorganism of the genus *Corynebacterium* according to any of claims 1 to 13 in a medium; and
- (ii) recovering putrescine or ornithine from the cultured microorganism or the cultured medium.

15. The method according to claim 14, wherein the microorganism of the genus *Corynebacterium* is *Corynebacterium glutamicum*.