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(54) **CORYNEBACTERIUM GLUTAMICUM VARIETY PRODUCING L-ARGININE AND METHOD FOR FABRICATING THE SAME**

UNTERART VON CORYNEBACTERIUM GLUTAMICUM ZUR ERZEUGUNG VON L-ARGININ AND UND VERFAHREN ZU IHRER HERSTELLUNG

VARIÉTÉ DE CORYNEBACTÉRIUM GLUTAMICUM PRODUISANT DE LA L-ARGININE ET SON PROCÉDÉ DE FABRICATION

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Description

Technical Field

[0001] The present invention relates to an L-arginine producing mutant strain, which produces L-arginine in a high yield to be used in medicinal and pharmaceutical industries, and a method for fabricating the same. In particular, the present invention relates to a polynucleotide comprising an *argD2* gene(*Ncgl2355*) which is a putative gene of acetylornithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum,* a polypeptide encoded by the polynucleotide, a recombinant vector comprising the polynucleotide, a transformant capable of producing L-arginine in a high yield, which is prepared by introducing the recombinant vector into an L-arginine producing host microorganism to overexpress the *argD2* gene, and a method for producing L-arginine by culturing the transformant.

Background Art

[0002] L-arginine is a free-form amino acid found in plant seeds or garlic. L-arginine has been widely used as an efficient additive in medicaments, food, or the like. L-arginine is useful as a drug for improving the hepatic function and brain function, and treating male sterility, and as an ingredient of multiple amino acid supplements. Also, L-arginine has been used as a food additive in fish cakes and health beverages, and has recently gained interest as a salt substitute for hypertension patients.

[0003] Conventional methods for L-arginine production by biological fermentation are based on the production of L-arginine directly from carbon and nitrogen sources. For example, L-arginine can be produced using a mutant strain derived from a glutamic acid-producing microorganism belonging to the genus *Brevibacterium* or *Corynebacterium* (Japanese Unexamined Patent Publication Nos. Sho57-163487, Sho60-83593 and Sho62-265988), or using an amino acid-producing microorganism, of which growth properties are improved through cell fusion (Japanese Unexamined Patent Publication No. Sho59-158185). Recently, it has been reported that L-arginine can be produced using a recombinant strain, of which an *argR* gene that participates in regulation of arginine biosynthesis is inactivated (US Patent Application No. 2002/0045223A1) and using a method for over-expressing an *argF* geneof arginine operon (Korean Patent Application No. 10-2004-107215).

[0004] In microorganisms, biosynthesis of L-arginine proceeds in eight enzymatic steps starting from the precursor L-glutamate and follows two different pathways, the linear pathway or the cyclic pathway.

[0005] In microorganisms belonging to the genus *Corynebacterium,* L-arginine is synthesized through the cyclic pathway from L-glutamate via N-acetylglutamate, N-acetylglutamyl phosphate, N-acetylglutamate semialdehyde, N-acetylornithine, ornithine, citrulline and argininosuccinate. These intermediates are synthesized through consecutive reactions catalyzed by enzymes such as glutamate N-acetyltransferase, N-acetylglutamate kinase, acetylglutamate semialdehyde dehydrogenase, acetylornithine aminotransferase, ornithine cabomoyltransferase, arginosuccinate synthase, and arginosuccinate lyase. These enzymes are encoded by *argJ, argB, argC, argD, argF, argG* and *argH* genes, respectively.

[0006] In order to produce L-arginine in a high yield, the present inventors have conducted studies on the enzymes involved in L-arginine biosynthesis for a long period time. They found that in the intermediate step of arginine biosynthesis, the enzymatic reaction involved in the conversion of N-acetylglutamate semialdehyde to N-acetylornithine is amplified to increase L-arginine flux, thereby improving the productivity of L-arginine.

[0007] A variety of aminotransferases are present in cells, and divided into four subgroups on the basis of their mutual structural relatedness. Subgroup I comprises aspartylornithine, alanine, tyrosine, histidiolphosphate, and phenylalanine aminotransferases, subgroup II comprises acetylornithine, ornithine, ω-amino acid, aminobutyrate, and phenylalanine aminotransferases, subgroup III comprises D-alanine and branched-chain amino acid aminotransferases, and subgroup IV comprises serine and phosphoserine aminotransferases (Perdeep K. MEHTA, et al, Eur. J. Biochem., 214, 549-561, 1993).

[0008] It has been known that in *Corynebacterium glutamicum,* an *argCJBDF* gene involved in arginine biosynthesis exists as an operon, and is regulated by feedback-inhibition due to arginine (Vehary Sakanyan, et al, Microbiology, 142: 9-108, 1996). Thus, there is a limit in producing L-arginine in a high yield.

Disclosure of Invention

Technical Problem

[0009] Accordingly, the present inventors have made an effort to develop strains capable of producing L-arginine in higher yield. They found that a microorganism transformed with an *argD2* gene, which is a putative gene having the same function as an *argD* gene encodingacetylornithine aminotransferases, overexpresses the *argD2* gene and produces L-arginine in higher yield than a parent strain, thereby completing the present invention.

Technical Solution

[0010] It is an object of the present invention to provide a polypeptide encoded by an *argD2* gene(*Ncgl2355*) that is a putative gene of acetylornithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum.*

[0011] It is another object of the present invention to provide a recombinant vector comprising a base sequence encoding the polypeptide.

[0012] It is still another object of the present invention to provide a transformant capable of producing L-arginine in a high yield, which is prepared by introducing the recombinant vector.

[0013] It is still another object of the present invention to provide a method for producing L-arginine by culturing the transformant. However, in particular, the present invention relates to a method of producing L-arginine as claimed hereafter. Preferred embodiments of the invention are set forth in the dependent claims.

Brief Description of the Drawings

[0014] Fig. 1 illustrates the construction of a recombinant plasmid pHC131T-*argD2*, comprising an *argD2* gene, *CJ1* promoter and $rrnB_1B_2$ terminator.

Best Mode for Carrying Out the Invention

[0015] In one embodiment, the present invention provides a polypeptide encoded by an *argD2* gene(*Ncgl2355*) which is a putative gene of acetylornithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum.* Preferably, an amino acid sequence of the polypeptide may be represented by SEQ ID NO. 1.

[0016] The *argD2* gene(*Ncgl2355*) which is a putative gene of acetylomithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum* is an aminotransferase classified into subgroup II according to genome based analysis (Alice C. McHardy, et al, J. Biotechnology, 104, 229-240, 2003), however, function of the protein encoded by the *argD2* has not been clearly identified. There is a little sequence homology between *argD* and *argD2* genes, however, both genes have the same motif, aminotransferase subgroup II. Therefore, it is inferred that the protein encoded by the *argD2* gene has similar function to acetylornithine aminotransferase that is encoded by the *argD* gene and required for arginine biosynthesis (www.genome.jp/kegg/).

[0017] It can be seen that the L-arginine producing strain transformed with a recombinant vector comprising the *argD2* gene produces L-arginine in higher yield than a parent strain.

[0018] Also provided is a polynucleotide encoding an amino acid sequence of SEQ ID NO. 1. Preferably, the base sequence of the polynucleotide may be represented by SEQ ID NO. 2. Further, also described is a polynucleotide having 70% or more homology with the base sequence of SEQ ID NO. 2, preferably 90% or more homology with the base sequence of SEQ ID NO. 2.

[0019] Also described is a recombinant vector comprising the polynucleotide. Preferably, the recombinant vector may comprise the polynucleotide represented by SEQ ID NO. 2, and may be a recombinant vector pHC131T-*argD2* prepared as described herein.

[0020] The recombinant vector may be easily fabricated by those skilled in the art according to any known method using DNA recombination technique. For example, the genomic DNA is isolated from the L-arginine producing strain, and PCR is performed using the isolated genomic DNA as a template to amplify the ORF region of *argD2* gene. To fabricate a recombinant vector for overexpression, *CJ1* promoter (Korean Patent No. 10-0620092) and $rrnB_1B_2$ terminator regions are amplified using pECCG117-CJ1 and *E.coli K-12* as a template, respectively. The *CJ1* promoter that is known as the upstream region of *Corynebacterium ammoniagenes* Hsp60 and strongly expressed in *Corynebacterium glutamicum,* and commercially available *rrnB1B2* terminator are used as a promoter and terminator, respectively. In this connection, the base sequence of *argD2* geneis analyzed by a conventional sequencing method. The promoter region, terminator region and *argD2* gene are cloned into a suitable plasmid or other cloning vectors, and transformed into suitable competent cells to fabricate a recombinant vector (Fig. 1).

[0021] In the fabrication of the recombinant vector, any vector expressed in prokaryotic or eukaryotic cells may be used as a cloning vector, and in a specific embodiment of the present invention, a plasmid pECCG117 (Han J.K., et al, Biotechnology letters, 13(10):721-726, 1991 or Korean Patent Publication No. 92-7401) is used. Further, the L-arginine producing strain encompasses all microorganisms capable of producing L-arginine, including prokaryotic or eukaryotic cells, preferably *Escherichia coli, coryneform bacteria,* and *Bacillus species* capable of producing L-arginine, and more preferably *Corynebacterium glutamicum* capable of producing L-arginine.

[0022] The present invention provides a transformant capable of producing L-arginine in a high yield, which is prepared by introducing the recombinant vector into an L-arginine producing host microorganism to overexpress the *argD2* gene.

[0023] Specifically, the host microorganism has a high DNA introduction efficiency and expression efficiency, and may be any microorganism capable of producing L-arginine, including prokaryotic or eukaryotic cells. The preferred Examples

thereof include any one selected from the group consisting of thespecies *Escherichia, Aerobacter, Schizosaccharomyces, Pichia, Kluyveromyces, Candida, Hansenula, Debaryomyces, Zygosaccharomyces, Mucor, Torulopsis, Methylobacter, Salmonella, Bacillus, Streptomyces, Pseudomonas, Brevibacterium, Magnetospirillum* and *Corynebacterium,* more preferably microorganisms belonging to the genus *Corynebactrium,* whichhave a resistance to L-arginine analogues and produce L-arginine, and most preferably *Corynebacterium glutamicum* ATCC21493 or ATCC21831. Examples of the L-arginine analogues include an alpha-amino acid canavanine found in Canavalia ensiformis and arginine hydroxamate.

[0024] In a specific embodiment of the present invention, the recombinant vector pHC131T-*argD2* is introduced into *Corynebacterium glutamicum* ATCC21493 and *Corynebacterium glutamicum* ATCC21831, which have a resistance to L-arginine analogues and produce L-arginine, so as to prepare transformed microorganisms CA06-0012 and CA06-0013. The transformed microorganisms CA06-0012 and CA06-0013 were deposited at the Korean Culture Center of Microorganisms (hereinafter, abbreviated to "KCCM" on December 13, 2006 under accession number KCMM10820P and KCMM10821P, respectively.

[0025] The transformed microorganisms can be easily prepared by those skilled in the art according to any known method. The term "transformation" as used herein means introducing DNA into a host cell so that DNA is replicable, either as an extra-chromosomal element or by chromosomal integration, that is, artificial genetic alteration by introducing a foreign DNA into a host cell. Examples thereof include a $CaCl_2$ precipitation, a Hanahan method that is an improved $CaCl_2$ method by using DMSO (dimethyl sulfoxide) as a reducing material, electroporation, calcium phosphate precipitation, protoplast fusion, agitation using silicone carbide fiber, Agrobacterium-mediated transformation, PEG-, dextran sulfate-, and lipofectamine-mediated transformation. In one embodiment of the present invention, the recombinant vector pHC131T-*argD2* was introduced into host cells by electroporation to prepare transformants, and a strain harboring the recombinant vector was selected using its antibiotic resistance.

[0026] To increase the L-arginine productivity of the transformant prepared according to the present invention, the *argD2* gene present in the chromosome of the transformant may be additionally subjected to expression or deletion by a conventional recombinant technique. It is known in the related art that its base sequence can be analyzed by a sequencing method using fluorescence.

[0027] In the biosynthetic pathway of L-arginine, ornithine is an intermediate of the metabolic pathway of arginine and is an important material in nitrogen metabolism along with the urea cycle. The CA06-0012 and CA06-0013 strains transformed by the method of the present invention are transformants overexpressing the *argD2* gene, prepared by the following method. The *argD2* gene encoding a putative protein having a function of acetylornithine aminotransferase, which is obtained by PCR from the chromosome of the L-arginine producing strain *Corynebacterium glutamicum* ATCC21831, is inserted into a vector, and introduced into the L-arginine producing strains, *Corynebacterium glutamicum* ATCC21493 and ATCC21831. It was found that the transformed CA06-0012 and CA06-0013 strains according to the present invention overexpress the *argD2* gene to increase the synthesis of N-acetylornithine, thereby activating the arginine biosynthetic pathway to produce L-arginine in a high yield.

[0028] Accordingly, in another embodiment, the present invention provides a method for producing L-arginine, comprising the step of culturing the transformant, preferably transformant represented by accession number KCCM10820P or KCCM10821P.

[0029] In the production method of L-arginine according to the present invention, the cultivation of the transformed L-arginine overexpressing microorganisms may be conducted in suitable media and under culture conditions known in the art. The culturing procedures can be readily adjusted by those skilled in the art according to the selected strain. Examples of the culturing procedures include batch type, continuous type and fed-batch type manners, but are not limited thereto. Various culturing procedures are disclosed in literature, for example, "Biochemical Engineering" (James M. Lee, Prentice-Hall International Editions, pp 138-176, 1991).

[0030] During cultivation, ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid, and sulfuric acid may be properly added to adjust the pH of the cultures. Defoaming agents such as fatty acid polyglycol ester may be properly added to reduce the formation of foams in cultures. Further, to maintain the cultures in aerobic states, oxygen or oxygen-containing gas (e.g., air) may be injected into the cultures. The cultures are maintained at 20 to 45°C and preferably at 25 to 40°C. The cultivation may be continued until a desired amount of L-arginine is obtained, and preferably for 10 to 160 hrs. The isolation of L-arginine from the culture broth may be performed by the conventional method known in the art. Examples thereof may include centrifugation, filtration, ion-exchange chromatography, and crystallization. For example, the cultures may be subjected to low-speed centrifugation to remove the biomass, and the supernatant may be separated by ion-exchange chromatography.

Mode for the Invention

[0031] Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are for the illustrative purpose only, and the invention is not intended to be limited by these Examples.

Example 1. Preparation of *argD2* gene. *CJ1* promoter and *rrnB$_1$B$_2$* terminator

[0032] To construct a recombinant vector pHC131T comprising an *argD2* gene that *is a* putative gene of acetylornithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum, CJ1* promoter that is strongly expressed in *Corynebacterium glutamicum,* and *rrnB$_1$B$_2$* terminator, a DNA fragment (1.371 Kb) including Open Reading Frame (hereinafter, abbreviated to "ORF") of *argD2* genewas obtained from the genomic DNA (gDNA) of L-arginine producing strain ATCC21831, and PCR was performed using pECCG117-CJ1 (Korean Patent Application No. 10-2004-107215) and the genome of *E.coli K-12 W3110* as a template to obtain the *CJ1* promoter (0.3 Kb) and the *rrnB$_1$B$_2$* Terminator (0.4 Kb), respectively.

Example 1-1. Amplification of DNA fragment including ORF of *argD2* gene

[0033] The genomic DNA (gDNA) was extracted from L-arginine producing strain ATCC21831 using a Genomic-tip system (QIAGEN, hereinafter the same). In order to amplify the DNA fragment (1.371 Kb) including ORF of *argD2* gene, polymerase chain reaction (hereinafter, abbreviated to "PCR") was performed using the gDNA as a template and a PTC-200 Peltier Thermal Cycler (MJ Research, USA, hereinafter the same). At this time, primers used in the amplification of the ORF region of *argD2* gene were as follows: SEQ ID NO. 7; 5'-tcccccgggggattggcatgaagggttac-3' and SEQ ID NO. 8; 5'-gctctagagcttagaacaacgccccagc-3'. PCR conditions included 25 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min. The PCR product was subjected to electrophoresis on a 1.0% agarose gel, and then a band of 1.3 kb was eluted from the gel.

Example 1-2. Amplification of *CJ1* promoter

[0034] To amplify the *CJ1* promoter, PCR was performed using pECCG117-CJ1 (Korean Patent Application No. 10-2004-107215) as a template and a PTC-200 Peltier Thermal Cycler. At this time, primers used in the amplification of *CJ1* promoter were as follows: SEQ ID NO. 3; 5'-cgggtaccaccgcgggcttattccattacat-3' and SEQ ID NO. 4; 5'-acgcgatatcttaatctcctagattgggtttc-3'. PCR conditions included 25 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 68°C for 30 sec. The PCR product was subjected to electrophoresis on a 1.0% agarose gel, and then a band of 0.3 kb was eluted from the gel.

Example 1-3. Amplification of *rrnB$_1$B$_2$* terminator

[0035] The genomic DNA (gDNA) was extracted from *E.coli K-12 W3110* using a Genomic-tip system. To amplify the *rrnB$_1$B$_2$* Terminator, PCR was performed using the gDNA as a template and a PTC-200 Peltier Thermal Cycler. At this time, primers used in the amplification of *rrnB$_1$B$_2$* terminator were as follows: SEQ ID NO. 5; 5'-gctctagagctgttttggcggatgaga-3' and SEQ ID NO. 6; 5'-ataagaatgcggccgccgcaaaaaggccatccgtcag-3'. PCR conditions are the same as in the amplification of *CJ1* promoter. The PCR product was subjected to electrophoresis on a 1.0% agarose gel, and then a band of 411 bp was eluted from the gel.

Example 2. Construction of recombinant plasmid

Example 2-1. Construction of recombinant plasmid pHC131T

[0036] A plasmid pECCG-117 (Han J.K., et al, Biotechnology letters, 13(10):721-726, 1991 or Korean Patent Publication No. 92-7401), which is an *E. coli/c. glutamicum* shuttle vector, was treated with restriction enzymes, EcoRV and KpnI, and then subjected to electrophoresis on a 0.8% agarose gel to elute a band of about 5.9 Kb. Further, the *CJ1* promoter prepared in Example 1-2 was treated with restriction enzymes, KpnI and EcoRV, and then isolated using a Quiaquick PCR purification kit (Qiagen, hereinafter the same).

[0037] Two DNA fragments were ligated using a Quick ligation kit (NEB, hereinafter the same) to prepare a recombinant plasmid pECCG1 17-CJ1. The recombinant plasmid pECCG117-CJ1 was treated with restriction enzymes, XbaI and Not1, and then subjected to electrophoresis on a 0.8% agarose gel to elute a band of about 6.2 Kb. The prepared pECCG 117-CJ1 and the *rrnB$_1$B$_2$* terminator prepared in Example 1-3 were ligated using a Quick ligation kit to obtain a recombinant plasmid pECCG117-CJ1-rrnB$_1$B$_2$ (about 6.6 Kb), which was designated as "pHC131T" in the present invention.

[0038] The prepared pHC131T plasmid was treated with EcoRV and XbaI, and then subjected to electrophoresis on a 1% agarose gel to elute a band of about 6.6 Kb from the gel.

Example 2-2. Construction of plasmid pHC131T-argD2

[0039] The PCR product of *argD2* gene prepared in Example 1-1 was treated with restriction enzymes, smaI and XbaI, and then subjected to electrophoresis on a 0.8% agarose gel to elute a band of about 1.3 Kb from the gel. The resultant was ligated with pHC131T prepared in Example 2-1 using a Quick ligation kit, so as to construct a recombinant plasmid of about 7.4 Kb (Fig. 1), which was designated as "pHC131T-*argD2"* in the present invention.

Example 3. Sequencing analysis of *argD2* gene

[0040] To analyze the base sequence of pHC131T-*argD2* prepared in Example 2-2, PCR was performed using 0.1 ug DNA of pHC131T-*argD2* as a template with 2 mM of a pair of primers of SEQ ID NOs. 3 and 6 and 1□ of a BigDyeTM Terminator Cycle Sequencing v2.0 Ready Reaction (PE Biosystems). PCR conditions included 25 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 2 min, followed by quenching at 4°C to terminate the reaction. The PCR product was subjected to electrophoresis on a 0.8% agarose gel, and then a DNA fragment of 2 kb was eluted from the gel.

[0041] This DNA fragment was subjected to sequencing analysis using the primer of SEQ ID NO. 3 on an ABI PRISM 3100 Genetic Analyzer™ (Applied Biosystems). An amino acid sequence encoded by the *argD2* gene of the present invention and a base sequence thereof are shown in SEQ ID NOs. 1 and 2, respectively.

Example 4. Preparation of transformant

[0042] The recombinant plasmid pHC131T-*argD2* prepared in Example 2-2 was introduced into L-arginine producing strains, ATCC 21493 and ATCC 21831 by electroporation to prepare transformants overexpressing the *argD2* gene, which were designated as CA06-0012 and CA06-0013, respectively. The transformed microorganisms CA06-0012 and CA06-0013 were deposited at the Korean Culture Center of Microorganisms (hereinafter, abbreviated to "KCCM") on December 13, 2006 under accession number KCMM10820P and KCMM10821P, respectively.

[0043] The strains and transformants were smeared on solid media containing 25 mg/L of Kanamycin (composition: 3.0 g/L of Beef extract, 5.0 g/L of peptone, hereinafter the same), and cultured at 30°C for 16 hrs. The selected colonies were subjected to a flask titer test as the following Example 5. As a result, it was found that the *argD2* - overexpressing transformant according to the present invention produced L-arginine in a high yield.

Example 5. Comparison of arginine production titer in Erlenmeyer flask

[0044] Transformants prepared in Example 4 and parent strains, ATCC21831 and ATCC21493 were smeared on solid media containing 25 mg/L of Kanamycin, and cultured at 30°C for 16 hrs to select 10 single colonies from each strain. The selected colonies were cultured in L-arginine seed media given in Table 1, and then evaluated for L-arginine productivity in an Erlenmeyer flask using titer media given in Table 1. The mean values of the L-arginine productivity were calculated and compared.

Table 1

Ingredients	L-argininеseed media	L-argininetiter media
Glucose	5%	4%
Bactopeptone	1%	-
Sodium chloride (NaCl)	0.25%	-
Yeast extract	1%	-
Biotin	3 □/L	200 □/L
Urea	0.4%	0.3%
pH	7.0	7.2
Ammonium sulfate ($(NH_4)_2SO_4$)	-	3%
Potassium Dihydrogen Phosphate (KH_2PO_4)	-	0.1%
Potassium Monohydrogen Phosphate (K_2HPO_4)	-	0.1%
Magnesium sulfate heptahydrate ($MgSO_47H_2O$)	-	0.025%

(continued)

Ingredients	L-argininеseed media	L-argininetiter media
CSL (corn steep liquor)	-	2.0%

[0045] The selected colonies were inoculated in the seed media and cultured in an incubator at 30°C for 16 hrs. 1 ml of the seed culture was inoculated in 24 ml of the titer media, and culturing was carried out at 30°C and 220 rpm for 72 hrs. The results of L-arginine production titer test for ATCC21831, ATCC21493 and transformants are given in Table 2.

[0046] As shown in Table 2, the arginine producing strains *Corynebacterium glutamicum* ATCC 21493 and ATCC 21831 exhibited L-arginine productivity of 0.9 g/L and 4.4 g/ L, respectively. Meanwhile, the *argD2*-overexpressing recombinant strains CA06-0012 and CA06-0013 of the present invention exhibited L-arginine productivity of 1.1 g/L and 4.9 g/L, respectively. It can be seen that the productivity of the recombinant strains CA06-0012 and CA06-0013 was increased by 0.2 g/L (22.2%) and 0.5 g/L (11.4%), as compared to each parent strain.

Table 2

Productivity	Strain			
	Parent strain (ATCC21493)	Transformant (CA06-0012)	Parent strain (ATCC21831)	Transformant (CA06-0013)
L-arginine (g/L)	0.9	1.1	4.4	4.9

[0047] It should be understood that the above Examples and Experimental Examples are not limitative, but illustrative in all aspects.

Industrial Applicability

[0048] The present invention provides a polynucleotide comprising an *argD2* gene(*Ncgl2355)* that is a putative gene of acetylornithine aminotransferase involved in arginine biosynthesis of *Corynebacterium glutamicum,* a polypeptide encoded by the polynucleotide, a recombinant vector comprising the polynucleotide, a transformant capable of producing L-arginine in a high yield, which is prepared by introducing the recombinant vector into an L-arginine producing host microorganism to overexpress the *argD2* gene, and a method for producing L-arginine by culturing the transformant. The transformant of the present invention overexpresses the *argD2* gene to produce L-arginine in a high yield, thereby being used in medicinal and pharmaceutical industries, and feed industry.

<110> CJ cheiljedang Corporation

<120> Corynebacterium glutamicum Variety Producing L-Arginine and Method for Fabricating the Same

<130> OPA08001/PCT

<150> KR10-2007-0005831
<151> 2007-01-18

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Arg Ala His Val Phe His Ser Trp Ser Ala Gln Asp Lys Ile Ser Pro
        35                  40                  45

Lys Val Trp Ala Ala Ala Glu Gly Ser Thr Leu Tyr Asp Phe Asp Gly
    50                  55                  60

Asn Ala Phe Ile Asp Met Gly Ser Gln Leu Val Ser Ala Asn Leu Gly
65                  70                  75                  80

His Asn Asn Pro Arg Leu Val Glu Ala Ile Gln Arg Gln Ala Ala Arg
                85                  90                  95

Leu Thr Asn Ile Asn Pro Ala Phe Gly Asn Asp Val Arg Ser Asp Val
            100                 105                 110

Ala Ala Lys Ile Val Ser Met Ala Arg Gly Glu Phe Ser His Val Phe
        115                 120                 125

Phe Thr Asn Gly Gly Ala Asp Ala Ile Glu His Ser Ile Arg Met Ala
    130                 135                 140

Arg Leu His Thr Gly Arg Asn Lys Ile Leu Ser Ala Tyr Arg Ser Tyr
145                 150                 155                 160

His Gly Ala Thr Gly Ser Ala Met Met Leu Thr Gly Glu His Arg Arg
                165                 170                 175

Leu Gly Asn Pro Thr Thr Asp Pro Asp Ile Tyr His Phe Trp Ala Pro
            180                 185                 190

Phe Leu His His Ser Ser Phe Phe Ala Thr Thr Gln Glu Glu Glu Cys
        195                 200                 205

Glu Arg Ala Leu Lys His Leu Glu Asp Val Ile Ala Phe Glu Gly Ala
    210                 215                 220

Gly Met Ile Ala Ala Ile Val Leu Glu Pro Val Val Gly Ser Ser Gly
225                 230                 235                 240

Ile Ile Leu Pro Pro Ala Gly Tyr Leu Asn Gly Val Arg Glu Leu Cys
                245                 250                 255

Asn Lys His Gly Ile Leu Phe Ile Ala Asp Glu Val Met Val Gly Phe
            260                 265                 270

Gly Arg Thr Gly Lys Leu Phe Ala Tyr Glu His Ala Gly Asp Asp Phe
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Gln Pro Asp Met Ile Thr Phe Ala Lys Gly Val Asn Ala Gly Tyr Ala
290                 295                 300

Pro Leu Gly Gly Ile Val Met Thr Gln Ser Ile Arg Asp Thr Phe Gly
305                 310                 315                 320

Ser Glu Ala Tyr Ser Gly Gly Leu Thr Tyr Ser Gly His Pro Leu Ala
                325                 330                 335

Val Ala Pro Ala Lys Ala Ala Leu Glu Ile Tyr Ala Glu Gly Glu Ile
            340                 345                 350

Ile Pro Arg Val Ala Arg Leu Gly Ala Glu Leu Ile Glu Pro Arg Leu
        355                 360                 365

Arg Glu Leu Ala Glu Glu Asn Val Ala Ile Ala Asp Val Arg Gly Ile
    370                 375                 380

Gly Phe Phe Trp Ala Val Glu Phe Asn Ala Asp Ala Thr Ala Met Ala
385                 390                 395                 400

Ala Gly Ala Ala Glu Phe Lys Glu Arg Gly Val Trp Pro Met Ile Ser
                405                 410                 415

Gly Asn Arg Phe His Ile Ala Pro Pro Leu Thr Thr Thr Asp Asp Glu
            420                 425                 430

Leu Val Ala Leu Leu Asp Ala Val Glu Ala Ala Ala Gln Ala Val Glu
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Leu Thr Phe Ala Gly Ala Leu Phe
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tccgcgcagg acaaaatcag ccccaaagta tgggcagctg ccgaaggttc cacgctgtac 180

gacttcgacg gcaacgcctt catcgacatg ggttcccaac ttgtctcggc aaacttaggc 240

cacaacaacc ctcgattagt tgaggcgatc cagcgccaag cagcccggtt gaccaacatc 300

aacccggctt tcggcaatga tgtgcgctct gatgttgctg caaagatcgt gtcgatggcc 360

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gcaggttacg ccccactcgg tggcatcgtg atgacccaat caatccgcga taccttcgga 960

tcagaggcat actccggcgg actcacctac tccggacacc cacttgcagt agcacccgcc 1020

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gccggtgctg cagaattcaa ggaacgcggc gtgtggccga tgatctccgg caaccgattc 1260

cacatcgcgc cgccgctgac caccactgat gacgaattgg tagcactgct ggacgcggtg 1320

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<210> 8
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28

Claims

1. A method for producing L-arginine, comprising the step of culturing a microorganism containing a recombinant vector comprising a polynucleotide encoding an argD2 polypeptide, wherein the argD2 polypeptide has an amino acid sequence consisting of SEQ ID NO. 1.

2. The method for producing L-arginine according to claim 1, wherein the polynucleotide consists of SEQ ID NO.2.

3. The method for producing L-arginine according to claim 1, wherein the microorganism belongs to the genus Corynebacterium.

4. The method for producing L-arginine according to claim 1, wherein the microorganism is identified by accession number KCCM10820P or KCCM10821 P.

Patentansprüche

1. Ein Verfahren zum Herstellen von L-Arginin, das den Schritt des Kultivierens eines Mikroorganismus, der einen rekombinanten Vektor enthält, welcher ein ein argD2-Polypeptid codierendes Polynukleotid beinhaltet, beinhaltet, wobei das argD2-Polypeptid eine Aminosäuresequenz aufweist, die aus SEQ ID NO. 1 besteht.

2. Verfahren zum Herstellen von L-Arginin gemäß Anspruch 1, wobei das Polynukleotid aus SEQ ID NO. 2 besteht.

3. Verfahren zum Herstellen von L-Arginin gemäß Anspruch 1, wobei der Mikroorganismus zur Gattung Corynebacterium gehört.

4. Verfahren zum Herstellen von L-Arginin gemäß Anspruch 1, wobei der Mikroorganismus durch die Zugangsnummer KCCM10820P oder KCCM10821 P identifiziert wird.

Revendications

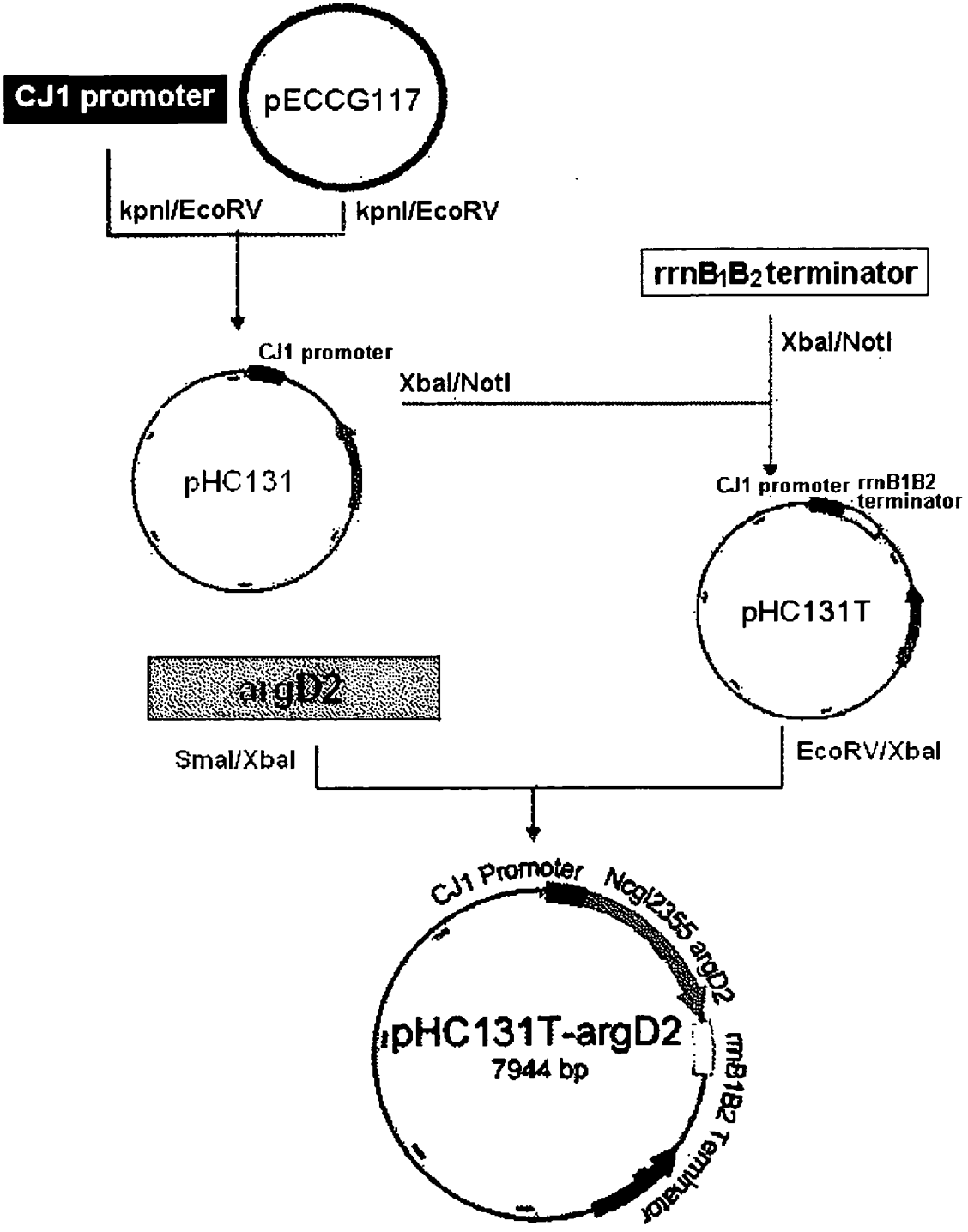
1. Une méthode pour produire de la L-arginine, comprenant l'étape de culture d'un micro-organisme contenant un vecteur recombinant comprenant un polynucléotide codant pour un polypeptide argD2, dans laquelle le polypeptide argD2 a une séquence d'acides aminés consistant en SEQ ID NO. 1.

2. La méthode pour produire de la L-arginine selon la revendication 1, dans laquelle le polynucléotide consiste en SEQ ID NO. 2.

3. La méthode pour produire de la L-arginine selon la revendication 1, dans laquelle le micro-organisme appartient au genre Corynebacterium.

4. La méthode pour produire de la L-arginine selon la revendication 1, dans laquelle le micro-organisme est identifié par le numéro d'accession KCCM10820P ou KCCM10821P.

[Fig. 1]


CJ1 promoter
pECCG117
kpnI/EcoRV
kpnI/EcoRV
rrnB1B2 terminator
XbaI/NotI
CJ1 promoter
XbaI/NotI
pHC131
CJ1 promoter rrnB1B2 terminator
pHC131T
argD2
SmaI/XbaI
EcoRV/XbaI
CJ1 Promoter
Ncgl2355 argD2
rrnB1B2 Terminator
pHC131T-argD2
7944 bp

REFERENCES CITED IN THE DESCRIPTION

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