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(56) Related Art
J. FERMENT TECH, 1984, 62, PP 569-575
J. FERMENT TECH, 1984, 62, PP 371-376
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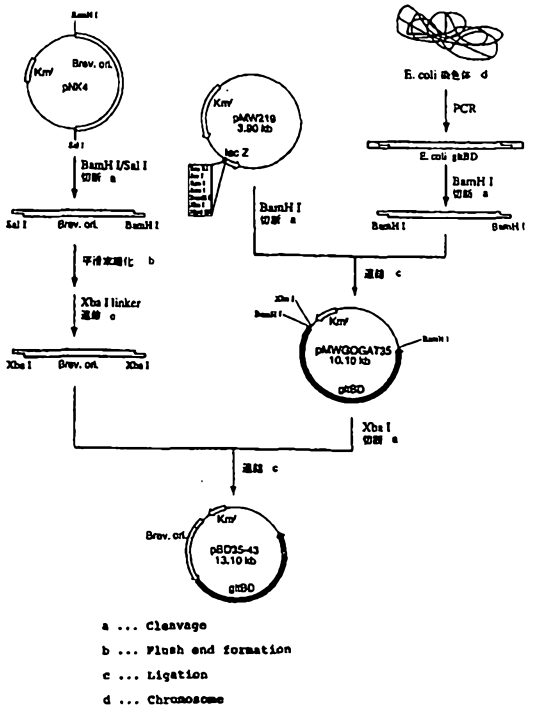
(54)Title: **PROCESS FOR PRODUCING L-GLUTAMIC ACID BY FERMENTATION METHOD**

(54)発明の名称 発酵法によるL－グルタミン酸の製造法

(57) **Abstract**

L-Glutamic acid is efficiently produced at a low cost by culturing a strain which belongs to the genus *Corynebacterium* , has an enhanced glutamine-oxoglutarate aminotransferase activity and is capable of producing L-Glutamic acid in a liquid medium to accumulate L-Glutamic acid in the culture medium, and harvesting the acid thus accumulated.



Abstract

L-glutamic acid is produced by cultivating, in a liquid medium, a bacterial strain belonging to the Corynebacterium which has an ability to produce L-glutamic acid, wherein glutamine-oxoglutarate amino transferase activity is enhanced in a cell of the bacterial strain, producing and accumulating L-glutamic acid in the culture medium, and recovering the L-glutamic acid from the medium.

Method for Producing L-Glutamic Acid by Fermentation

Technical Field

The present invention relates to a method for producing L-glutamic acid by fermentation. L-glutamic acid is an important amino acid which is used for food, clinical drugs and others.

Background Art

Conventionally, L-glutamic acid has been mainly produced by fermentative methods using so-called L-glutamic acid-producing coryneform bacteria which belong to the genus Brevibacterium, Corynebacterium or Microbacterium, or mutants thereof (Amino Acid Fermentation, Gakkai Shuppan Center, 195-215, (1986)). As fermentative methods for producing L-glutamic acid using other bacterial strains, those which use microorganisms including Bacillus, Streptomyces, and Penicillium (U.S. Patent 3,220,929), and those which use microorganisms including the genera Pseudomonas, Arthrobacter, Serratia, and Candida (U.S. Patent 3,563,857) are known. Although productivity for L-glutamic acid has been much increased by using conventional methods, development of a method for

producing L-glutamic acid which is more effective and inexpensive is required in order to satisfy further increasing demand.

L-glutamic acid is biosynthesized from α-ketoglutaric acid which is an intermediate in the citric acid cycle existing in cell of microorganisms, and there are two different biosynthetic pathways to form L-glutamic acid from α-ketoglutaric acid by assimilation of ammonium ion. One is the pathway through which, in the presence of ammonium ion with a high concentration, L-glutamic acid is synthesized by catalysis of glutamate dehydrogenase (hereinafter referred to as "GDH"), and the other is the pathway (GS/GOGAT pathway) through which L-glutamic acid is synthesized by glutamine synthetase (hereinafter referred to as "GS") which catalyzes reaction from L-glutamic acid and ammonium ion to glutamine, and by glutamine-oxoglutaric acid amino transferase (also called as "glutamate synthase", and hereinafter referred to as "GOGAT") which catalyzes L-glutamic acid synthetic reaction in which two molecules of L-glutamic acid is produced from one molecule of glutamine, that has been synthesized by GS, and one molecule of α-ketoglutaric acid. So far, L-glutamic acid production by a bacterial strain GDH pathway of which has been enhanced through elevation of the GDH activity has been reported, but L-glutamic acid

production by bacterial strain GS/GOGAT pathway of which is enhanced has not been known.

Disclosure of the Invention

One aspect of the present invention is to breed bacterial strains having high L-glutamic acid productivity, and to provide a method for producing L-glutamic acid which is more effective and inexpensive, in order to satisfy further increasing demand of L-glutamic acid.

The present inventors has conducted studies on methods for producing L-glutamic acid using bacterial strains being enhanced GOGAT activity which is one of enzymes that participate in the GS/GOGAT pathway, and catalyzes production of L-glutamic acid. As a result, the inventors has found that a bacterial strain having an ability to produce L-glutamic acid which is enhanced GOGAT activity has higher L-glutamic acid productivity, and thus completed the present invention.

That is, the present invention provides a bacterial strain belonging to the genus Corynebacterium which has an ability to produce L-glutamic acid, wherein glutamine-oxoglutarate amino transferase activity is enhanced in a cell of the bacterial strain. The enhancement of the glutamine-oxoglutarate amino

transferase activity may be caused through amplifying the copy number of a gene encoding glutamine-oxoglutarate amino transferase. Alternatively, the enhancement of the glutamine-oxoglutarate amino transferase activity may be also caused through alteration of expression regulation sequence of a gene encoding the enzyme. The glutamine-oxoglutarate amino transferase may be derived from bacterium belonging to the genus Escherichia or Corynebacterium.

Further, the present invention provides a gene encoding glutamine-oxoglutarate amino transferase which comprises a nucleotide sequence corresponding to at least nucleotide numbers of 565 to 6614 of a nucleotide sequence depicted in SEQ ID NO: 7.

Also, the present invention provides a method for producing L-glutamic acid by fermentation, comprising the steps of cultivating, in a liquid medium, a bacterial strain belonging to the genus Corynebacterium which has an ability to produce L-glutamic acid, wherein glutamine-oxoglutarate amino transferase activity is enhanced in a cell of said bacterial strain, producing and accumulating L-glutamic acid in the culture medium, and recovering the L-glutamic acid from the medium.

The present invention will be explained in detail below.

(1) Bacteria belonging to the genus Corynebacterium having an ability to produce L-glutamic acid

Bacteria belonging to the genus Corynebacterium as referred to herein are a group of microorganisms defined in Bergey's Manual of Determinative Bacteriology, 8th Ed., p. 599 (1974). The bacteria are aerobic, Gram-positive, non-acid-fast bacilli not having the ability to sporulate, and include bacteria which had been classified as bacteria belonging to the genus Brevibacterium but have now been unified into the genus Corynebacterium [see Int. J. Syst. Bacteriol., 41, 255 (1981)] and also include bacteria of the genus Brevibacterium and Microbacterium which are closely related to the genus Corynebacterium. Of such bacteria belonging to the genus Corynebacterium, those mentioned below, which are known as L-glutamic acid-producing bacteria, are most preferred for use in the present invention.

Corynebacterium acetoacidophilum

Corynebacterium acetoglutamicum

Corynebacterium callunae

Corynebacterium glutamicum

Corynebacterium lilium (Corynebacterium glutamicum)

Corynebacterium melassecola

Brevibacterium divaricatum (Corynebacterium glutamicum)

Brevibacterium lactofermentum (Corynebacterium glutamicum)

Brevibacterium saccharolyticum

Brevibacterium immariophilium

Brevibacterium roseum

Brevibacterium flavum (Corynebacterium glutamicum)

Brevibacterium thiogenitalis

Specifically, the following strains of these bacteria are exemplified:

Corynebacterium acetoacidophilum	ATCC 13870
Corynebacterium acetoglutamicum	ATCC 15806
Corynebacterium callunae	ATCC 15991
Corynebacterium glutamicum	ATCC 13032
Corynebacterium glutamicum	ATCC 13060
Brevibacterium divaricatum	ATCC 14020
Brevibacterium lactofermentum	ATCC 13869
Corynebacterium lilium	ATCC 15990
Corynebacterium melassecola	ATCC 17965
Brevibacterium saccharolyticum	ATCC 14066
Brevibacterium immariophilium	ATCC 14068
Brevibacterium roseum	ATCC 13825
Brevibacterium flavum	ATCC 13826
Brevibacterium thiogenitalis	ATCC 19240

These strains can be obtained from the American

Type Culture Collection (ATCC). A registration number has been assigned to each strain of bacteria. Based on the registration number, anyone can obtain the corresponding strain of bacteria from ATCC. The registration numbers of the strains of bacteria deposited in ATCC are described in the ATCC catalog.

To produce L-glutamic acid using above described bacteria belonging to the genus Corynebacterium, any of the following means is effective.

1. The biotin concentration in the medium is made suboptimal. Refer to S. Okumura, T. Tsugawa, T. Tsunoda and A. Kitai, Nippon Nogeikagaku Kaishi, 36, 197-203 (1962).

2. A surfactant is added to the medium provided that a sufficient amount of biotin is present. Refer to I. Shiio, H. Otsuka and N. Atsuya, J. Biochem., 53, 333-340 (1963); K. Takinami, H. Okada and T. Tsunoda, Agr. Biol. Chem., 27, 853-863 (1963).

3. Penicillin is added to the medium provided that a sufficient amount of biotin is present. Refer to U.S. Patent 3,080,297; Japanese Patent Publication No. 37-1695 (1962); M. Shibui, T. Kurima, S. Okabe and T. Osawa, Amino Acid and Nucleic Acid, 17, 61-65 (1968).

Also, L-glutamic acid can be produced by a method using mutants derived from the above-described bacteria belonging to the genus Corynebacterium. As examples of

the mutant, the following may be mentioned:

Brevibacterium lactofermentum AJ 12745 (FERM-BP 2922); see U.S. Patent No. 5,272,067.

Brevibacterium lactofermentum AJ 12746 (FERM-BP 2923); see U.S. Patent No. 5,272,067.

Brevibacterium flavum AJ 12747 (FERM-BP 2924); see U.S. Patent No. 5,272,067.

Corynebacterium glutamicum AJ 12478 (FERM-BP 2925); see U.S. Patent No. 5,272,067.

Corynebacterium glutamicum ATCC 21492.

In the present invention, it is most preferable to use L-glutamic acid-producing bacterial strains as described above.

(2) Enhancement of GOGAT activity

GOGAT activity in a bacterial cell can be enhanced by constructing recombinant DNA by ligating a gene fragment that codes for GOGAT with a vector that functions in bacteria belonging to the genus Corynebacterium, transforming a host strain of bacterium belonging to the genus Corynebcterium which has an ability to produce L-glutamic acid by introducing the recombinant DNA to the strain. GOGAT is a heterooligomer comprising large subunit and small

subunit each of which is coded by gltB gene and gltD gene, respectively. As a result of increase of copy number of the gene that codes for GOGAT (hereinafter referred to as "gltBD gene") in cells of the transformants, GOGAT activity is enhanced.

As to the gltBD gene, the gene of bacteria belonging to the genus Corynebacterium and also the gene derived from other organisms including Esherichia coli can be used. Nucleotide sequence of the gltBD gene derived from bacteria belonging to the genus Corynebacterium has not been known, but nucleotide sequences of gltBD genes of Esherichia coli K-12 (Gene, 60, 1-11 (1987)) and yeast (Saccharomyces cerevisiae, GenBank accession No. X89221) has been already clarified. Therefore, it is possible to synthesize primers based on the nucleotide sequences of these gltBD genes, and to obtain the gltBD gene of microorganisms such as Esherichia coli K-12 and Brevibacterium lactofermentum ATCC 13869 by the PCR method by using chromosomal DNA prepared from these microorganisms as a template. Such primers may be exemplified by the primers shown in SEQ ID Nos: 3 to 6. The nucleotide sequence of gltBD gene of Brevibacterium lactofermentum ATCC 13869 isolated in Example described later is shown in SEQ ID NO: 7. The gltBD gene of Brevibacterium lactofermentum is novel.

As for plasmids which are used for gene cloning, any plasmid that is replicable in cells of microorganism such as Esherichia may be used, and pBR322, pTWV228, pMW119 and pUC19 are concretely exemplified.

The vector functioning in bacterium belonging to the genus Corynebacterium as referred to herein is, for example, a plasmid which is autonomously replicable in bacteria belonging to the genus Corynebacterium. Specific examples of the vector are mentioned below.

pAM 330 see Japanese Patent Application Laid-Open No. 58-67699 (1983)

pHM 1519 see Japanese Patent Application Laid-Open No. 58-77895 (1983)

pAJ 655 see Japanese Patent Application Laid-Open No. 58-192900 (1983)

pAJ 611 see Japanese Patent Application Laid-Open No. 58-192900 (1983)

pAJ 1844 see Japanese Patent Application Laid-Open No. 58-192900 (1983)

pCG 1 see Japanese Patent Application Laid-Open No. 57-134500 (1982)

pCG 2 see Japanese Patent Application Laid-Open No. 58-35197 (1983)

pCG 4 see Japanese Patent Application Laid-Open No. 57-183799 (1982)

pCG 11 see Japanese Patent Application Laid-Open

In order to prepare recombinant DNA by ligating the gltBD gene which encodes GOGAT and a vector which can function in a cell of bacterium belonging to the genus Corynebacterium, the vector is digested by restriction enzyme(s) corresponding to the termini of the gltBD gene. Ligation is generally performed by using a ligase such as T4 DNA ligase.

To introduce the recombinant DNA prepared as described above to bacterium belonging to the genus Corynebacterium, any known transformation methods can be employed. For instance, employable are a method of treating recipient cells with calcium chloride so as to increase the permeability of DNA, which has been reported for Escherichia coli K-12 [see Mandel, M. and Higa, A., J. Mol. Biol., 53, 159 (1970)]; and a method of preparing competent cells from cells which are at the growth phase followed by introducing the DNA thereinto, which has been reported for Bacillus subtilis [see Duncan, C.H., Wilson, G.A. and Young, F.E., Gene, 1, 153 (1977)]. In addition to these, also employable is a method of making DNA-recipient cells into the protoplast or spheroplast which can easily take up recombinant DNAs followed by introducing the recombinant DNA into the cells, which is known to be applicable to Bacillus

subtilis, actinomycetes and yeasts [see Chang, S. and Choen, S.N., Molec. Gen. Genet., 168, 111 (1979); Bibb, M.J., Ward, J.M. and Hopwood, O.A., Nature, 274, 398 (1978); Hinnen, A., Hicks, J.B. and Fink, G.R., Proc. Natl. Sci., USA, 75, 1929 (1978)].

The method of transformation used in embodiments of the present invention is the electric pulse method (refer to Japanese Patent Publication Laid-Open No. 2-207791).

Enhancement of GOGAT activity can also be achieved by introducing multiple copies of the gltBD gene into the chromosomal DNA of the above-described host strains. In order to introduce multiple copies of the gltBD gene in the chromosomal DNA of bacterium belonging to the genus Corynebacterium, the homologous recombination is carried out using a sequence whose multiple copies exist in the chromosomal DNA as targets. As sequences whose multiple copies exist in the chromosomal DNA, repetitive DNA, inverted repeats exist at the end of a transposable element can be used. Also, as disclosed in Japanese Patent Publication Laid-Open No. 2-109985, it is possible to incorporate the gltBD gene into transposon, and allow it to be transferred to introduce multiple copies of the gltBD gene into the chromosomal DNA. By either method, the number of copies of the gltBD gene within cells of the transformant strain increases, and

as a result, GOGAT activity is enhanced.

Other than the above-described gene amplification, enhancement of GOGAT activity can also be achieved by substituting the expression regulation sequence such as promoter of the gltBD gene with a more potent one. For example, lac promoter, trp promoter, trc promoter, tac promoter, and P_R promoter and P_L promoter of lambda phage are known as potent promoters. By substituting the promoter inherent in gltBD gene with these promoters, the expression of gltBD gene is enhanced, thereby enhancing GOGAT activity.

(3) Production of L-glutamic acid using bacterial strains of the present invention

By using a bacterial strain belonging to the genus Corynebacterium which has an ability to produce L-glutamic acid, wherein GOGAT activity is enhanced in a cell of said bacterial strain, L-glutamic acid can be produced by an ordinary method which uses a common nutrient culture medium containing carbon source, nitrogen source, inorganic salts and other minor organic nutrients such as amino acids and vitamins as necessary. Both of synthetic and natural medium can be used. Any carbon or nitrogen source used for the medium can be employed if it can be used in the bacterial strain to be cultivated.

As carbon sources, sugars such as glucose, glycerol, fructose, sucrose, maltose, mannose, galactose, hydrolysate of starch, and molasses are used, and other organic acids such as acetic acid and citric acid are also used alone or in combination with other carbon sources.

As nitrogen sources, ammonia, ammonium salts such as ammonium sulphate, ammonium carbonate, ammonium chloride, ammonium phosphate and ammonium acetate or nitrate may be used.

As minor organic nutrients, amino acids, vitamins, fatty acids, nucleic acids, and further peptone, casamino acids, yeast extract and soy bean protein hydrolysate which contain these nutrients may be used, and in case of using an auxotrophic mutant which needs amino acids or others for its growth, necessary nutrients should be supplemented.

As inorganic salts, phosphates, magnesium salts, calcium salts, iron salts, manganese salts and the like are used.

As to the cultivation method, it is performed under an aerobic condition while fermentation temperature is controlled at between 20 and 45°C, and pH at between 5 and 9. If the pH value lowers during cultivation, calcium carbonate may be added or the culture medium is neutralized with alkaline substance such as ammonia gas.

By thus cultivating for about ten hours to four days, a significant amount of L-glutamic acid is accumulated in the culture medium.

As to the method for recovering L-glutamic acid from the culture medium after the cultivation, any known method for recovery can be used. For example, the product can be collected by the concentration crystallization method after removal of cell from the culture medium, or by ion-exchange chromatography or the like.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Brief Description of Drawing

Fig. 1 shows the process for construction of plasmid pBD35-43.

Description of Preferred Embodiments

The present invention will be more specifically explained below with reference to Examples.

Example 1

(1) Cloning of gltBD gene of Esherichia coli K-12

Nucleotide sequence of the gltBD gene of Esherichia coli K-12 has already been known (Gene, 60, 1-11 (1987)). Based on the reported nucleotide sequence,

primers shown by SEQ ID NOs: 1 and 2 in the Sequence Listing were synthesized and the gltBD gene was amplified according to the PCR method by using the chromosomal DNA of JM109 strain (produced by Takara Shuzo Co., Ltd.) derived from Esherichia coli K-12 as a template.

Among the synthesized primers, SEQ ID NO: 1 corresponds to a sequence ranging from the 57th to 96th nucleotides in the Figure of nucleotide sequence of the gltBD gene described in Gene, 60, 6 (1987), but the 77th and 78th nucleotides were changed from A to G in the primer, and the recognition site for the restriction enzyme BamHI has been inserted. SEQ ID NO: 2 corresponds to a sequence ranging from the 6261st to 6290th nucleotides in the Figure of nucleotide sequence of the gltBD gene described in Gene, 60, 7 (1987), but the 6380th nucleotide T was changed to G, the 6282nd nucleotide A was changed to T, and the 6284th nucleotide A was changed to C in the primer, thus the recognition site for the restriction enzyme BamHI has been inserted. Also the nucleotide sequence shown in SEQ ID NO: 2 is a sequence which is the oposite strand of the nucleotide sequence ranging from the 6261st to 6290th nucleotides described in the Figure of nucleotide sequence in Gene, 60, 7 (1987), and is directed from the 5' end.

Preparation of chromosomal DNA of Esherichia coli

K-12 was performed according to the ordinary method (Seibutsu-kogaku Jikken-sho, edited by Nihon Seibutsu Kougaku-kai, 97-98, Baifu-kan, (1992)). Also, the PCR reaction was performed by using standard reaction conditions described in PCR Technology (Edited by Henry Ehrich, Stockton Press, 1989, page 8).

After the obtained PCR product was purified using the ordinary method, it was treated with a restriction enzyme BamHI, and ligated with pMW219 (produced by Nippon Gene Co., Ltd.), which had been digested with BamHI, using a ligation kit (produced by Takara Shuzo Co., Ltd.), and then transformation was performed using competent cells of Esherichia coli JM109 (produced by Takara Shuzo Co., Ltd.). Then, the cells were plated on L medium (10 g/l Bacto-trypton, 5 g/l Bacto-yeast extract, 15 g/l NaCl, 15 g/l agar, pH 7.2) containing 10 μg/ml of IPTG (isopropyl-β-D-thiogalactopyranoside), 40 μg/ml of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and 25 μg/ml of kanamycin and cultivated over night, then formed white colonies were picked up and isolated as single colonies to obtain transformants.

Plasmids were prepared from the transformants using the alkaline method (Seibutsu-kogaku Jikken-sho, edited by Nihon Seibutsu Kogaku-kai, 105, Baifu-kan, (1992)), and after that, a restriction map of DNA fragment which had been inserted into vectors was prepared, and based

on the comparison with reported restriction map of the gltBD gene, a plasmid in which DNA fragment having the restriction map same as the reported map had been inserted was named as pMWGOGAT35.

Further, in order to confirm that the gltBD gene was expressed, pMWGOGAT35 was introduced in Esherichia coli PA340 strain which lacked gdh and gltB and had L-glutamic acid requirement (E. coli Genetic stock center (Yale University, U.S.A.)) using the electroporation method. As a result, the transformant in which pMWGOGAT35 was introduced lost the L-glutamic acid requirement, thereby expression of the gltBD gene was confirmed.

(2) Construction of a plasmid having the gltBD gene of Esherichia coli K-12 and a replication origin of bacterium belonging to the genus Corynebacterium

Process of construction of plasmid pBD35-43 which has the gltBD gene of Esherichia coli K-12 and a replication origin of bacterium belonging to the genus Corynebacterium is shown in Fig. 1. Concretely, plasmid pHK4 (Japanese Patent Application Laid-Open No. 5-7491) which carries the replication origin (Japanese Patent Application Laid-Open No. Hei 5-7491) derived from plasmid pHM1519 which is autonomously replicable in bacterium belonging to the genus Corynebacterium (Agric.

Biol. Chem., 48, 2901-2903 (1984)) was digested with restriction enzymes BamHI and KpnI to obtain a gene fragment including the replication origin, and the obtained fragment was blunt-ended using DNA blunting kit (produced by Takara Shuzo Co., Ltd., Blunting kit), and was then introduced at the XbaI site in the plasmid pMWGOGAT35 to which cloned gltBD gene of Esherichia coli K-12 strain had been inserted using XbaI linker (produced by Takara Shuzo Co., Ltd.). This plasmid was designated as pBD35-43.

(3) Introduction of pBD35-43 to wild strains of bacteria belonging to the genus Corynebacterium AJ12036 and AJ13029 and estimaion of the obtained transformants by cultivation

Wild strains of bacteria belonging to the genus Corynebacterium AJ12036 (Agric. Biol. Chem., 51, 93-100 (1987)) and AJ13029 were transformed with plasmid pBD35-43 using the electric pulse method (refer to Japanese Patent Publication Laid-Open No. 2-207791), and thus transformants were obtained. The transformant AJ12036/pBD35-43 which was obtained by introducing the plasmid pBD35-43 into the wild strain AJ12036 was cultivated to produce L-glutamic acid under biotin restriction condition as follows. Cells of AJ12036/pBD35-43 strain cultivated on CM2B medium plate

containing 25μg/ml of kanamycin were inoculated into culture medium in which 80 g of glucose, 1 g of KH_2PO_4, 0.4 g of $MgSO_4 \cdot 7H_2O$, 30 g of $(NH_4)_2SO_4$, 0.01 g of $FeSO_4 \cdot 7H_4O$, 0.01 g of $MnSO_4 \cdot 7H_2O$, 15 ml of soybean hydrolysate, 200 μg of thiamine hydrochloride, 60 μg of biotin, 25 mg of kanamycin, and 50 g of $CaCO_3$ were added in one liter of pure water (adjusted at pH 8.0 using KOH), and the cells were cultivated with shaking at 31.5 °C until sugar in the medium was consumed. Obtained culture was inoculated in the medium of the same composition except that biotin and kanamycin were not added (hereinafter referred to as "biotin restriction medium") at the amount of 5%, then cultivated with shaking at 31.5 °C until sugar in the medium was consumed. As a control, AJ12036 strain was cultured in the same manner as mentioned above. After the completion of cultivation, amount of accumulated L-glutamic acid in the culture medium was measured using Biotech Analyzer AS-210 produced by Asahi Chemical Industry Co., Ltd. Obtained results are shown in Table 1.

Table 1

Bacterial Strains	Accumulation of L-glutamic acid (g/L)
AJ 12036	34.3
AJ 12036/pBD35-43	36.8

Also, using a transformant AJ13029/pBD35-43 which had been obtained by introducing the plasmid pBD35-43 into AJ 13029 strain, cultivation for L-glutamic acid production was performed as follows. Cells of AJ13029/pBD35-43 strain cultured on CM2B medium plate containing 25 µg/ml of kanamycin were inoculated into culture medium in which 30 g of glucose, 1 g of KH_2PO_4, 0.4 g of $MgSO_4 \cdot 7H_2O$, 30 g of $(NH_4)_2SO_4$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.01 g of $MnSO_4 \cdot 7H_2O$, 15 ml of soy bean hydrolysate, 200 µg of thiamine hydrochloride, 60 µg of biotin, 25 mg of kanamycin, and 50 g of $CaCO_3$ were added in one liter of pure water (adjusted at pH 8.0 using KOH), and the culture medium was subjected to cultivation with shaking at 31.5 °C until sugar in the medium was consumed. Obtained culture was inoculated in the medium of the same composition at the amount of 5%, then cultivated with shaking at 37.0 °C until sugar in the medium was consumed. As a control, AJ12029 strain was cultured in the same manner as mentioned above. After the completion of cultivation, amount of accumulated L-glutamic acid in the culture medium was measured using Biotech Analyzer AS-210 produced by Asahi Chemical Industry Co., Ltd. Obtained results are shown in Table 2. AJ12029 strain is a strain which is described in WO96/06180 and has temperature-sensitivity

for biotin activity repressing substance. This strain was deposited in National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (zip code: 305-8566, 1-3 Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, Japan) under the Budapest Treaty. The deposition number of the strain is FERM BP-5189.

Table 2

Bacterial Strains	Accumulation of L-glutamic acid (g/L)
AJ 13029	16.5
AJ 13029/pBD35-43	17.7

Based on the above results, it was clarified that L-glutamic acid-producing bacterium which belong to the genus Corynebacterium and have enhanced GOGAT activity via introduction of the gltBD gene showed increased yield of L-glutamic acid.

Example 2

(1) Cloning of the gltBD gene of Brevibacterium lactofermentum ATCC13869

It is desirable to use the gltBD gene originated from bacterium belonging to the genus Corynebacterium in

the case of fermentative production of L-glutamic acid using bacterium belonging to the genus Corynebacterium.

Oligonucleotides shown in SEQ ID NOs: 3 and 4 were synthesized according to the nucleotide sequence assumed from the amino acid sequence of the selected region of gltB gene products which are highly homologous between Escherichia coli and yeast. Chromosome DNA of was prepared from Brevibacterium lactofermentum ATCC 13869 using Bacterial Genomic DNA Purification Kit (produced by Advanced Genetic Technologies Corp.). Using the above oligonucleotides as primers and the chromosomal DNA as a template, PCR was performed under the standard reaction conditions described in "PCR Technology" (edited by Henry Ehrich, Stockton Press, 1989, page 8). As a result of agarose gel electrophoresis of the PCR product, it was revealed that a DNA fragment of approximately 1.4 kbp was amplified. Both termini of obtained DNA was sequenced using oligonucleotides of SEQ ID Nos: 3 and 4. Sequencing was performed using DNA Sequencing Kit (produced by Applied Biosystems Co., Ltd.) according to the Sanger method (J. Mol. Biol., 143, 161 (1980)). Determined nucleotide sequence was translated into amino acids, and compared the sequence with the amino acid sequences deduced from gltB genes of Esherichia coli and yeast. As a result, because of high homology, it was concluded that the DNA fragment

amplified by the PCR was a part of the gltB gene of Brevibacterium lactofermentum ATCC 13869. Then the chromosomal DNA of Brevibacterium lactofermentum ATCC13869 prepared by the above-described method was digested with EcoRI, BamHI, HindIII, PstI and SalI (produced by Takara Shuzo Co., Ltd.) by the ordinary method and was analyzed by Southern hybridization using the amplified DNA fragment as a probe and the DIG DNA Labeling and Detection Kit (produced by Boeringer Mannheim). As a result, it was found that an approximately 14 kb fragment cleaved by HindIII hybridizes with the probe DNA. Therefore, HindIII fragment of chromosomal DNA of Brevibacterium lactofermentum ATCC13869 prepared by the ordinary method was subjected to the agarose gel electrophoresis, and DNA fragments with approximately 10 kb or larger were recovered using glass powder. The recovered DNA fragment was ligated to vector pMW219 (produced by Nippon Gene Corporation) that had been cleaved with the restriction enzyme HindIII (produced by Takara Shuzo Co., Ltd.) using the Ligation kit (produced by Takara Shuzo Co., Ltd.). Competent cells of Esherichia coli JM109 (produced by Takara Shuzo Co., Ltd.) were transformed by the ligation mixture. Transformants were plated on L medium (10 g/ml of Bacto-Tripton, 5 g/l of Bacto-Yeast extract, 15 g/l of NaCl , 15 g/l of agar, pH

7.2) containing 10 μg/ml of IPTG (isopropyl-β-D-thioglactopyronoside), 40 μg/ml of X-Gal (5-bromo-4-chloro-3-indryl-β-D-galactoside) and 25 μg/ml of kanamycin, and incubated overnight. Thus formed white colonies were picked up and isolated to single colonies, and approximately 1,000 transformants were obtained. Using obtained transformants, plasmids were prepared by the alkaline method (Seibutsu-Kogaku Jikken-sho, edited by Nihon Seibutsu-kogaku Gakkai, 105, Baifu-kan, (1992)). PCR was performed using synthetic oligonucleotides having nucleotide sequences shown in SEQ ID NOs: 5 and 6 which were synthesized according to the sequenced portion in the DNA sequence which was used as a probe, and using above plasmid as a template and primers, respectively, under the above-described condition. Then, the transformants which gave an amplified fragment of approximately 1.3 kbp, the size of which is same with the DNA fragment that was amplified when PCR was performed using above primers and using chromosome of Brevibacterium lactofermentum ATCC 13869 as the template, were selected.

(2) Determination of nucleotide sequence of the gltBD gene of Brevibacterium lactofermentum ATCC13869

Plasmid DNA prepared from the transformants obtained in (1) by the alkaline method contained a DNA

fragment of approximately 14 kbp derived from chromosome of Brevibacterium lactofermentum ATCC13869. In the same manner with the above-described method, nucleotide sequence of the gltBD gene contained in the DNA fragment of approximately 14 kbp derived from chromosome of Brevibacterium lactofermentum ATCC 13869 was determined.

Within the thus determined nucleotide sequence, that of AatII fragment which contains gltBD gene is shown in SEQ ID NO: 7. From the nucleotide sequence, two open reading frame was presumed to exist, and amino acid sequences of the translation products that were deduced from the nucleotide sequence were also shown in SEQ ID NO: 7. That is, the gene which encode two proteins having the amino acid sequences shown in SEQ ID NP: 7 is the gltBD gene of Brevibacterium lactofermentum ATCC 13869. In the sequence, nucleotide numbers 565 to 5094 corresponds to the gltB gene, and nucleotide numbers 5097 to 6614 corresponds to the gltD gene. Amino acid sequences coded by the gltB gene and the gltD gene are shown in SEQ ID Nos: 8 and 9, respectively in this order. Incidentally, since the methionine residue located on the N-termini of the proteins are derived from ATG which is the initiation codon, the residue usually has no relation with the function of proteins in temselves, and it is well known that it will be removed by function of peptidase after translation. Accordingly,

in case of the above-described proteins, there is a possibility that the methionine residue may be removed.

The nucleotide sequences and amino acid sequences were compared homology with known sequences. Employed data bases were EMBL and SWISS-PROT. As a result, it was revealed that the DNA shown in SEQ ID NO: 7 is a novel gene in bacteria belonging to the genus Corynebacterium which has homology with the gltBD genes of Esherichia coli, yeast, or the like, which had already reported.

(3) Preparation of a plasmid carrying the gltBD gene of Brevibacterium lactofermentum ATCC 13869 and the replication origin of bacterium belonging to the genus Corynebacterium

In order to study the effect of amplification of the gltBD gene of Brevibacterium lactofermentum ATCC 13869, a plasmid which carried the gltBD gene of Brevibacterium lactofermentum ATCC 13869 and a replication origin of bacterium belonging to the genus Corynebacterium was constructed. Specifically, the plasmid DNA containing the approximately 14 kbp DNA fragment derived from chromosome of Brevibacterium lactofermentum ATCC 13869 which had been prepared from the transformants obtained in (1) by the alkalile method was cleaved with the restriction enzyme AatII to obtain

DNA fragment containing the gltBD gene of Brevibacterium lactofermentum ATCC 13869, and obtained fragment was blunt-ended with the DNA blunting kit (produced by Takara Shuzo Co., Ltd., Blunting kit), followed by being inserted into the site of restriction enzyme SmaI within plasmid pVC7 which carried the replication origin derived from plasmid pAM330 which can autonomously replicate in bacterium belonging to the genus Corynebacterium (Japanese Patent Application Laid-Open No. Sho 58-67699). This plasmid was designated as pVCGOGAT. The plasmid pVC7 was constructed by ligating pHSG399, a vector for E. coli (Cm^r; Takeshita, S. et al., Gene, 61, 63-74 (1987)) with pAM330, a cryptic plasmid of Brevibacterium lactofermentum. pAM330 was prepared from Brevibacterium lactofermentum ATCC 13869 strain. pHSG399 was digested with a restriction enzyme resulting one cleavage site, AvaII (produced by Takara Shuzo), blunt-ended by using T4 DNA polymerase, and ligated with pAM330 having been digested with HindIII (produced by Takara Shuzo) and blunt-ended by using T4 DNA polymerase. pVC7 is autonomously replicable in both E. coli and Brevibacterium lactofermentum and has a multiple cloning site originating from pHSG399 and lacZ'.

Further, in order to confirm that the gltBD gene was expressed, pVCGOGAT was introduced into Esherichia

coli PA340 strain which lacks gdh and gltB and has L-glutamic acid requirement (E. coli Genetic stock center (Yale University, U.S.A.)) by the electroporation method. As a result, the transformant strain carrying pVCGOGAT did not show L-glutamic acid requirement any longer, and expression of the gltBD gene was thus confirmed.

(4) Introduction of pVCGOGAT into wild strain of bacterium belonging to the genus Corynebacterium AJ 12036 and estimation of cultivation

Wild strain of bacterium belonging to the genus Corynebacterium AJ 12036 (Agric. Biol. Chem., 51, 93-100 (1987)) was transformed with plasmid pVCGOGAT by the electric pulse method (Japanese Patent Publication Laid-Open No. Hei 2-207791) to obtain a transformant. The transformant AJ 12036/pVCGOGAT which was obtained by introducing plasmid pVCGOGAT into wild strain AJ 12036 was cultivated for L-glutamic acid production under biotin restriction condition as follows. Cells of AJ 12036/pVCGOGAT strain obtained by cultivating on CM2B medium plate containing 5 mg/ml of chloramphenicol were inoculated in culture medium in which 80 g of glucose, 1 g of KH_2PO_4, 0.4 g of $MgSO_4 \cdot 7H_2O$, 30 g of $(NH_4)_2SO_4$, 0.01 g of $FeSO_4 \cdot 7H_4O$, 0.01 g of $MnSO_4 \cdot 7H_2O$, 15 ml of soybean hydrolysate, 200 μg of thiamine hydrochloride, 60 μg of

biotin, 10 mg of cholramphenicol, and 50 g of $CaCO_3$ were added in one liter of pure water (pH was adjusted at 8.0 with KOH) and cultivated with shaking at 31.5 °C until sugar in the culture medium was consumed.

Obtained culture was inoculated at amount of 5% to the medium of the same composition except that biotin and chloramphenicol were not added (hereinafter referred to as "biotin restriction medium"), and cultivated with shaking at 31.5 °C until sugar in the culture medium was consumed. As a control, a bacterial strain which was prepared by introducing pVC7 into AJ 12036 strain using the above-described method was cultivated in the same method as described above. After cultivation, amount of accumulated L-glutamic acid in the medium was measured using the Biotech Analyzer AS-210 (produced by Asahi Chemical Industry Co., Ltd.). The results are shown in Table 3.

Table 3

Bacterial Strains	Accumulation of L-glutamic acid (g/L)
AJ 12036/pVC7	33.6
AJ 12036/pVCGOGAT	37.0

Industrial Applicability

According to the method of the present invention, productivity of L-glutamic acid in bacterial strains which belong to the genus Corynebacterium, have enhanced GOGAT activity and are capable of producing L-glutamic acid can be increased, and thereby making L-glutamic acid production more inexpensive and effectively. And, gltBD gene derived from bacterium belonging to the genus corynebacteium was obtained and effectively used to the glutamate production in bacterium belonging to the genus corynebacteium.

EDITORIAL NOTE - NO.85615/98

The following sequence listing is part of the description.

SEQUENCE LISTING

<110> AJINOMOTO CO., INC.

<120> Method for Producing L-Glutamic Acid by Fermentation

<130> OP778

<140> PCT/JP98/03535
<141> 1998-08-07

<150> JP 9-216906
<151> 1997-08-12

<160> 9

<170> PatentIn Ver. 2.0

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Met Lys Pro Gln Gly Leu Tyr Asn Pro
1 5
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Ala His Glu His Asp Ala Cys Gly Val Ala Phe Ile Ala Asp Ile His
10 15 20 25
ggt cga ccc agc cgc agc att gtt gat cgt gca ctt gag gcg ctt cgc 687
Gly Arg Pro Ser Arg Ser Ile Val Asp Arg Ala Leu Glu Ala Leu Arg
30 35 40
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Asn Ile Asp His Arg Gly Ala Ala Gly Ala Glu Lys Asn Thr Gly Asp

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        60                  65                  70
tct ggc att gag ctt cct gag gca ggg gag tat gcc act ggt att gcg   831
Ser Gly Ile Glu Leu Pro Glu Ala Gly Glu Tyr Ala Thr Gly Ile Ala
    75                  80                  85
ttc ttg cct cgc ggt cgc atg gcg atg atg gat gct cag aag gaa att   879
Phe Leu Pro Arg Gly Arg Met Ala Met Met Asp Ala Gln Lys Glu Ile
90                  95                  100                 105
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Glu Arg Ile Ala Lys Gln Glu Gly Ala Asp Val Leu Gly Trp Arg Met
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Pro Ser Phe Ala Gln Ile Phe Leu Thr Val Pro Gly Lys Ser Gly Glu
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    155                 160                 165
ctg ggc acc acc aat ggt cgc gat acg gtg tat ttc ccg tcg cta tct   1119
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170                 175                 180                 185
tca cgc acc atc att tac aaa ggc atg ttg acc act ctg cag ctt gag   1167
Ser Arg Thr Ile Ile Tyr Lys Gly Met Leu Thr Thr Leu Gln Leu Glu
                190                 195                 200
ggc ttc ttt gag gat ctg ggt gat gct cgc ctg gag tcg gcc att gct   1215
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            205                 210                 215
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Ile Val His Ser Arg Phe Ser Thr Asn Thr Phe Pro Ser Trp Pro Leu
        220                 225                 230
gcg cac ccg tac cgt ttc gtt gcc cac aac ggt gag atc aac act gtg   1311
Ala His Pro Tyr Arg Phe Val Ala His Asn Gly Glu Ile Asn Thr Val
    235                 240                 245
cgt ggc aat gaa aac tgg atg cgc gcc cgc gag gcg ctt atc aaa aac   1359
Arg Gly Asn Glu Asn Trp Met Arg Ala Arg Glu Ala Leu Ile Lys Asn
250                 255                 260                 265
gac aag ctg ggc aat ttg agc agc gtg ctg cct atc tgc acc ccg gag   1407
Asp Lys Leu Gly Asn Leu Ser Ser Val Leu Pro Ile Cys Thr Pro Glu
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ggc tcg gat acc gcg cgt ttc gac gag gct ttg gag ctt ttg cac ctg   1455
Gly Ser Asp Thr Ala Arg Phe Asp Glu Ala Leu Glu Leu Leu His Leu
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ggc gga tac tca ctt ccg cat gct gtt gcg atg atg atc cct cag gcg   1503
Gly Gly Tyr Ser Leu Pro His Ala Val Ala Met Met Ile Pro Gln Ala
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tgg gaa cac aac aag acg ctg agc cct gag ctg cgt gat ttc tac gaa   1551
Trp Glu His Asn Lys Thr Leu Ser Pro Glu Leu Arg Asp Phe Tyr Glu
    315                 320                 325
tac cac tct tgt ctg atg gag cca tgg gat ggt cct gca gcg ctg gca   1599
Tyr His Ser Cys Leu Met Glu Pro Trp Asp Gly Pro Ala Ala Leu Ala
330                 335                 340                 345
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Arg Pro Gly Arg Ile Thr Ile Thr Asp Ser Gly Leu Val Val Met Ala
            365                 370                 375
tct gaa tcg gga gtg ttg gac ttg agg gag gag agc gtc gta aag cgt   1743
Ser Glu Ser Gly Val Leu Asp Leu Arg Glu Glu Ser Val Val Lys Arg
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act cgc gta cag cct gga cgc atg ttc ctt gtt gac acg gcc gag ggt   1791
Thr Arg Val Gln Pro Gly Arg Met Phe Leu Val Asp Thr Ala Glu Gly
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cgc atc gtt gaa gac gag gaa atc aag cag aaa tta agc gaa gcg cag   1839
Arg Ile Val Glu Asp Glu Glu Ile Lys Gln Lys Leu Ser Glu Ala Gln
410                 415                 420                 425
cca tat ggt gag tgg att cgc gat aat ttt gtg cat ctg gat cgt ctg   1887
Pro Tyr Gly Glu Trp Ile Arg Asp Asn Phe Val His Leu Asp Arg Leu
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cct cag aca cgc tac aac tac atg gcg cac tct cgt gct gtg ttg cgt   1935
Pro Gln Thr Arg Tyr Asn Tyr Met Ala His Ser Arg Ala Val Leu Arg
            445                 450                 455
cag cgt gtt ttc gga atc act gaa gaa gat gtg gat ttg ttg ctg ctg   1983
Gln Arg Val Phe Gly Ile Thr Glu Glu Asp Val Asp Leu Leu Leu Leu
        460                 465                 470
ccg atg gcc cgc cag ggt gct gag gcg att ggt tcc atg ggt tcg gat   2031
Pro Met Ala Arg Gln Gly Ala Glu Ala Ile Gly Ser Met Gly Ser Asp
    475                 480                 485
acg cca att gcg gcg cta tcc cag cga cca cgc atg ctt tat gat ttc   2079
Thr Pro Ile Ala Ala Leu Ser Gln Arg Pro Arg Met Leu Tyr Asp Phe
490                 495                 500                 505
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Phe Ala Gln Arg Phe Ala Gln Val Thr Asn Pro Pro Leu Asp Ser Ile
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cgc gaa aag cct gtg acc agc atg ttc act ttg ttg ggt gcg cag tct   2175
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        540                 545                 550
tcg ccg atc att gat aac cat gag ctg gcc acc ttg atc aat gcc aac   2271
Ser Pro Ile Ile Asp Asn His Glu Leu Ala Thr Leu Ile Asn Ala Asn
    555                 560                 565
gcg cat ggt gag tgg gat tcc ttt ggt gct gct gta att tct ggt ttg   2319
Ala His Gly Glu Trp Asp Ser Phe Gly Ala Ala Val Ile Ser Gly Leu
570                 575                 580                 585
tac cca gtg gct cac cat ggt gcc ggc atg aag gct gcg att gct cgt   2367
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Leu Leu Leu Thr Ser Ala Val His Gln Tyr Leu Val Gln Gln Arg Thr
    635                 640                 645
cgt acc cag tgc tcc ctg gtg gtg gaa tcc ggc gat gcc cgc gag gtt   2559
Arg Thr Gln Cys Ser Leu Val Val Glu Ser Gly Asp Ala Arg Glu Val
650                 655                 660                 665
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His His Leu Ala Met Leu Ile Gly Phe Gly Ala Asp Ala Ile Asn Pro
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Tyr Met Ala Phe Glu Thr Ile Asp Glu Leu Arg Met Lys Gly Gln Leu
            685                 690                 695
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acc act ggt gtg ctg aag gtg atg tcc aag atg ggc att gca acg gtg   2751
Thr Thr Gly Val Leu Lys Val Met Ser Lys Met Gly Ile Ala Thr Val
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Ser Ser Tyr Arg Gly Ala Gln Leu Ala Asp Val Thr Gly Leu His Gln
730                 735                 740                 745
gat ctc ctg gac aac tac ttc ggt ggc att gct tca cca att tct ggt   2847
Asp Leu Leu Asp Asn Tyr Phe Gly Gly Ile Ala Ser Pro Ile Ser Gly
                750                 755                 760
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Ile Gly Leu Asp Glu Val Ala Ala Asp Val Glu Ala Arg His Arg Ser
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        780                 785                 790
ggt ggt gaa tac aag tgg cgc cgc gaa ggt gaa tat cac ctg ttc aac   2991
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cca gaa acc atc ttc aag ctg cag cat gca act cgt tct ggc agc tac   3039
Pro Glu Thr Ile Phe Lys Leu Gln His Ala Thr Arg Ser Gly Ser Tyr
810                 815                 820                 825
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Glu Ile Phe Lys Asp Tyr Thr Arg Lys Val Asp Asp Gln Ser Thr Arg
                830                 835                 840
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Leu Gly Thr Ile Arg Gly Leu Phe Glu Phe Ser Thr Asp Arg Lys Pro
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        860                 865                 870
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    875                 880                 885
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Val Leu Ala Ile Ala Met Asn Arg Leu Gly Gly Met Ser Asn Ser Gly
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gaa ggt ggc gag gac gcc cgc cga ttc gat gtg gaa ccc aac ggt gac   3327
Glu Gly Gly Glu Asp Ala Arg Arg Phe Asp Val Glu Pro Asn Gly Asp
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tgg aag cgc tct gcc att aag cag gtg gcc tcg gga cgt ttc ggc gtg   3375
Trp Lys Arg Ser Ala Ile Lys Gln Val Ala Ser Gly Arg Phe Gly Val
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acc agc cac tac ttg aac aac tgc acc gat att cag atc aag atg gca   3423
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cag ggc gca aag ccc ggt gaa ggt ggc cag ctg cca cca aac aag gtg   3471
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    955                 960                 965
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                990                 995                1000
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Ser Lys Ala His Ala Asp Val Val Leu Ile Ser Gly His Asp Gly Gly
    1035                1040                1045
act ggc gca tct cct ttg acc tcc ctg aag cat gcc ggt ggt cca tgg   3759
Thr Gly Ala Ser Pro Leu Thr Ser Leu Lys His Ala Gly Gly Pro Trp
1050                1055                1060                1065
gag ttg ggc ttg gct gaa acc cag caa acg ttg ctg ctc aac ggc ctg   3807
Glu Leu Gly Leu Ala Glu Thr Gln Gln Thr Leu Leu Leu Asn Gly Leu
                1070                1075                1080
cgt gat cgt att cgc gtg cag tgc gat ggt cag ctg aaa act ggc cga   3855
Arg Asp Arg Ile Arg Val Gln Cys Asp Gly Gln Leu Lys Thr Gly Arg
            1085                1090                1095
gac gta gtt atc gca gct ctg ctc ggt gcc gaa gaa ttc ggt ttc gcc   3903
Asp Val Val Ile Ala Ala Leu Leu Gly Ala Glu Glu Phe Gly Phe Ala
        1100                1105                1110
acc gca ccg ctg gtg gtt gaa ggc tgc atc atg atg cgc gtc tgc cac   3951
Thr Ala Pro Leu Val Val Glu Gly Cys Ile Met Met Arg Val Cys His
    1115                1120                1125
ctg gac acc tgc ccg gtg ggt atc gct acc cag aac ccg gat ttg cgt   3999
Leu Asp Thr Cys Pro Val Gly Ile Ala Thr Gln Asn Pro Asp Leu Arg
1130                1135                1140                1145
tcc aag ttc acc ggc aag gct gaa cac gtg gtc aac ttc ttc acc ttc   4047
Ser Lys Phe Thr Gly Lys Ala Glu His Val Val Asn Phe Phe Thr Phe
                1150                1155                1160
atc gcc cag gaa gtc cgt gag tac ttg gca cag ctt ggt ttc cgc tct   4095
Ile Ala Gln Glu Val Arg Glu Tyr Leu Ala Gln Leu Gly Phe Arg Ser
            1165                1170                1175
att gat gaa gcc gta gga caa gcc cag gtg ctg cgt aag cgt tcc gga   4143
Ile Asp Glu Ala Val Gly Gln Ala Gln Val Leu Arg Lys Arg Ser Gly
        1180                1185                1190
atc cca gct gat tcc cgc gca gca cac ctg gat ttg agc cca att ttc   4191
Ile Pro Ala Asp Ser Arg Ala Ala His Leu Asp Leu Ser Pro Ile Phe
    1195                1200                1205
cat cgc cca gaa act cca cac ttc cca act cag gat gtg cgt tgc acc   4239
His Arg Pro Glu Thr Pro His Phe Pro Thr Gln Asp Val Arg Cys Thr
1210                1215                1220                1225
aag acc cag gaa cac agc cta gaa aaa gcc ctg gac aac gca ttt att   4287
Lys Thr Gln Glu His Ser Leu Glu Lys Ala Leu Asp Asn Ala Phe Ile
                1230                1235                1240
gat aag gct tcg gac aca atc acc cgt gcc gca gcg ggt gtg gaa acc   4335
Asp Lys Ala Ser Asp Thr Ile Thr Arg Ala Ala Ala Gly Val Glu Thr
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            1245                1250                1255
agc att gtt att gat agc tcc atc agc aac gtc aac cgt tca gtt ggc   4383
Ser Ile Val Ile Asp Ser Ser Ile Ser Asn Val Asn Arg Ser Val Gly
        1260                1265                1270
acg atg ctg ggt tct gca gtc agc cgc gtg gct ggt gcc caa ggt ttg   4431
Thr Met Leu Gly Ser Ala Val Ser Arg Val Ala Gly Ala Gln Gly Leu
    1275                1280                1285
cca gac ggc acc atc acc ttg aat ctt caa ggc tgc gcc ggt aac tcc   4479
Pro Asp Gly Thr Ile Thr Leu Asn Leu Gln Gly Cys Ala Gly Asn Ser
1290                1295                1300                1305
ttt ggc gcg ttc atc cca cga ggc atc acc atc aac ctc acc ggc gat   4527
Phe Gly Ala Phe Ile Pro Arg Gly Ile Thr Ile Asn Leu Thr Gly Asp
                1310                1315                1320
gcc aat gac ttt gtg ggc aag gga tta tct ggc gga aag att gtg atc   4575
Ala Asn Asp Phe Val Gly Lys Gly Leu Ser Gly Gly Lys Ile Val Ile
            1325                1330                1335
aag cct tcc gct cag gct ccg aag cag ctg aag aac aat cca aat atc   4623
Lys Pro Ser Ala Gln Ala Pro Lys Gln Leu Lys Asn Asn Pro Asn Ile
        1340                1345                1350
att gcc gga aac gtg ctt gga tac ggc gca acc agt ggt gaa ttg ttc   4671
Ile Ala Gly Asn Val Leu Gly Tyr Gly Ala Thr Ser Gly Glu Leu Phe
    1355                1360                1365
att cgt ggc cag gtc ggc gaa cgt ttc tgc gtc cgt aac tct ggc gcc   4719
Ile Arg Gly Gln Val Gly Glu Arg Phe Cys Val Arg Asn Ser Gly Ala
1370                1375                1380                1385
acc gca gtg gtt gaa ggt atc gga aac cac ggt tgt gag tac atg act   4767
Thr Ala Val Val Glu Gly Ile Gly Asn His Gly Cys Glu Tyr Met Thr
                1390                1395                1400
ggc ggc cga gtc ctg gtt ttg ggc ccg gtt ggt gag aac ttt ggt gcc   4815
Gly Gly Arg Val Leu Val Leu Gly Pro Val Gly Glu Asn Phe Gly Ala
            1405                1410                1415
ggc atg tct ggt ggc att gca tac ctg gct aat tcc ccg gac cta aac   4863
Gly Met Ser Gly Gly Ile Ala Tyr Leu Ala Asn Ser Pro Asp Leu Asn
        1420                1425                1430
cag aag atc aat ggc gaa ttg gtg gat gtt gtt cca ctg agc gct gac   4911
Gln Lys Ile Asn Gly Glu Leu Val Asp Val Val Pro Leu Ser Ala Asp
    1435                1440                1445
gat ctg acg tgg gct gat gag ctc att gct cgc cac cgc gaa ctc acc   4959
Asp Leu Thr Trp Ala Asp Glu Leu Ile Ala Arg His Arg Glu Leu Thr
1450                1455                1460                1465
gga tcc gag acc aag ctg cgt gca caa gat ttg gtg aaa atc atg ccg   5007
Gly Ser Glu Thr Lys Leu Arg Ala Gln Asp Leu Val Lys Ile Met Pro
                1470                1475                1480
cgc gat ttc caa aaa gta ctc aac atc atc gaa acg gcc cac gct gag   5055
Arg Asp Phe Gln Lys Val Leu Asn Ile Ile Glu Thr Ala His Ala Glu
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ggc caa gac cca gca atc aag atc atg gag gca gtg agc ta atg gcc    5102
Gly Gln Asp Pro Ala Ile Lys Ile Met Glu Ala Val Ser    Met Ala
        1500                1505                1510     1
gac cca caa gga ttc atc aaa tac tcc cga cgc gag cct gca cac cgc   5150
Asp Pro Gln Gly Phe Ile Lys Tyr Ser Arg Arg Glu Pro Ala His Arg
          5                  10                  15
ccg gtc ccg ctg cgc ctc atg gac tac tcc gag gtc tac gaa aag gca   5198
Pro Val Pro Leu Arg Leu Met Asp Tyr Ser Glu Val Tyr Glu Lys Ala
     20                  25                  30
ccg gca ggt cag atc gag gaa cag gct gcc cgc tgc atg gat tgc ggt   5246
Pro Ala Gly Gln Ile Glu Glu Gln Ala Ala Arg Cys Met Asp Cys Gly
 35                  40                  45                  50
gtc ccg ttc tgc cac gaa ggc tgc cca ctg ggc aac atc atc cct gag   5294
Val Pro Phe Cys His Glu Gly Cys Pro Leu Gly Asn Ile Ile Pro Glu
                 55                  60                  65
tgg aat gat ctg gta cgc caa ggt cgg tgg aag gaa gcc tac gat cgc   5342
Trp Asn Asp Leu Val Arg Gln Gly Arg Trp Lys Glu Ala Tyr Asp Arg
             70                  75                  80
ctg cac gcg acc aac aat ttc ccc gag ttc acc ggc cgt ttg tgc ccc   5390
Leu His Ala Thr Asn Asn Phe Pro Glu Phe Thr Gly Arg Leu Cys Pro
         85                  90                  95
gca ccc tgc gaa ggc gcc tgc gtg ctc ggc atc aac gat gat tct gtc   5438
Ala Pro Cys Glu Gly Ala Cys Val Leu Gly Ile Asn Asp Asp Ser Val
    100                 105                 110
acc atc aaa aac gtt gag ctg gaa atc gtc gaa aaa gca ttc cgc gaa   5486
Thr Ile Lys Asn Val Glu Leu Glu Ile Val Glu Lys Ala Phe Arg Glu
115                 120                 125                 130
ggc tgg gtt cag cct gtc gtc gca tcc atg tcc acc ggc ctg tcc gta   5534
Gly Trp Val Gln Pro Val Val Ala Ser Met Ser Thr Gly Leu Ser Val
                135                 140                 145
gcc gtc gtc ggc tcc ggt ccc gct ggc ctt gcc gcc gcg cag cag ctc   5582
Ala Val Val Gly Ser Gly Pro Ala Gly Leu Ala Ala Ala Gln Gln Leu
            150                 155                 160
acc cgc gca ggt cac agc gtg acc gtc ttc gaa cgc gac gac cgc ctc   5630
Thr Arg Ala Gly His Ser Val Thr Val Phe Glu Arg Asp Asp Arg Leu
        165                 170                 175
ggc ggc ctc atg cgc tac ggc gtg cca gaa tac aaa atg gaa aac cgc   5678
Gly Gly Leu Met Arg Tyr Gly Val Pro Glu Tyr Lys Met Glu Asn Arg
    180                 185                 190
tgg atc gac cgc cgc atc gag caa atg gaa gca gag ggc aca act ttc   5726
Trp Ile Asp Arg Arg Ile Glu Gln Met Glu Ala Glu Gly Thr Thr Phe
195                 200                 205                 210
cag gta ggc acc tcg cca cgc gcc gct gaa cta gcg ctt ttc gac gcg   5774
Gln Val Gly Thr Ser Pro Arg Ala Ala Glu Leu Ala Leu Phe Asp Ala
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                215                 220                 225
atc ctc ctc gca acc ggc acc cca gtg gcc cgc gaa ctc tca gtt cca   5822
Ile Leu Leu Ala Thr Gly Thr Pro Val Ala Arg Glu Leu Ser Val Pro
            230                 235                 240
ggc cac gat ctc aac ggc atc cat gcg gca atg gat tac ctc acc gcc   5870
Gly His Asp Leu Asn Gly Ile His Ala Ala Met Asp Tyr Leu Thr Ala
        245                 250                 255
caa aac cgc atc aat gaa ggc gac ggt gaa gtc tct cca atc aac gcc   5918
Gln Asn Arg Ile Asn Glu Gly Asp Gly Glu Val Ser Pro Ile Asn Ala
    260                 265                 270
aaa ggc aag aaa gtt gtc atc atc ggt ggc ggc gac acc ggc acc gac   5966
Lys Gly Lys Lys Val Val Ile Ile Gly Gly Gly Asp Thr Gly Thr Asp
275                 280                 285                 290
tgc ttt ggt aca gcg ctg cgc caa gga gcg gaa tca gtc acc caa ttt   6014
Cys Phe Gly Thr Ala Leu Arg Gln Gly Ala Glu Ser Val Thr Gln Phe
                295                 300                 305
gat atc cgc ccc cgc gct cct ttc cag cgc gcc gat tcc acc cca tgg   6062
Asp Ile Arg Pro Arg Ala Pro Phe Gln Arg Ala Asp Ser Thr Pro Trp
            310                 315                 320
ccg atg tac ccc aac ctc ttc cgc acc gca acg gct cac gaa gaa ggc   6110
Pro Met Tyr Pro Asn Leu Phe Arg Thr Ala Thr Ala His Glu Glu Gly
        325                 330                 335
gaa tac atc atc act ggc gat gaa tca gcc gat gaa atc gca gcc ctg   6158
Glu Tyr Ile Ile Thr Gly Asp Glu Ser Ala Asp Glu Ile Ala Ala Leu
    340                 345                 350
ggc ctc gcc gaa cgc gcc gca ggc tcc acg ctt ggt gaa cgt aaa ttt   6206
Gly Leu Ala Glu Arg Ala Ala Gly Ser Thr Leu Gly Glu Arg Lys Phe
355                 360                 365                 370
gct gtc aac acc gtg gaa ttc cac ggc aac aac ggc cac gtc acc gga   6254
Ala Val Asn Thr Val Glu Phe His Gly Asn Asn Gly His Val Thr Gly
                375                 380                 385
ctc acc ggc aac caa atc cga gtt gtc aac ggc aaa cgt gaa cca atc   6302
Leu Thr Gly Asn Gln Ile Arg Val Val Asn Gly Lys Arg Glu Pro Ile
            390                 395                 400
gaa ggc acc gaa ttt ccc ttc gaa gca gac ctc gtt ctc gtc gca ctc   6350
Glu Gly Thr Glu Phe Pro Phe Glu Ala Asp Leu Val Leu Val Ala Leu
        405                 410                 415
gga ttc acc ggc gca gaa caa ggc gga ttg gca cac gaa cta ggc gta   6398
Gly Phe Thr Gly Ala Glu Gln Gly Gly Leu Ala His Glu Leu Gly Val
    420                 425                 430
ggt ttc gac gac cga gga cgc atc ctc cgc gat tcc gaa tac cgc agc   6446
Gly Phe Asp Asp Arg Gly Arg Ile Leu Arg Asp Ser Glu Tyr Arg Ser
435                 440                 445                 450
ccc acc aac tcc cgc gtt tac atc gca ggc gac aac ggc cgt ggc caa   6494
Pro Thr Asn Ser Arg Val Tyr Ile Ala Gly Asp Asn Gly Arg Gly Gln
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tcc ctg atc gtg tgg gca atc gcc gaa ggc cgc gca tgc gcc gca gct   6542
Ser Leu Ile Val Trp Ala Ile Ala Glu Gly Arg Ala Cys Ala Ala Ala
            470                 475                 480
atc gac gcc gac ctc atg ggt gaa act gca ctt cca gta gca gtt gca   6590
Ile Asp Ala Asp Leu Met Gly Glu Thr Ala Leu Pro Val Ala Val Ala
        485                 490                 495
cca cag gac gtg ccg ctg gct gtc taggaggatt ggcgccaaga accactcgaa  6644
Pro Gln Asp Val Pro Leu Ala Val
    500                 505
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<212> PRT
<213> Brevibacterium lactofermentum

<400> 8

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Gly Val Ala Phe Ile Ala Asp Ile His Gly Arg Pro Ser Arg Ser Ile
             20                  25                  30
Val Asp Arg Ala Leu Glu Ala Leu Arg Asn Ile Asp His Arg Gly Ala
         35                  40                  45
Ala Gly Ala Glu Lys Asn Thr Gly Asp Gly Ala Gly Ile Leu Met Gln
     50                  55                  60
Ile Pro Asp Gly Phe Tyr Arg Glu Val Ser Gly Ile Glu Leu Pro Glu
 65                  70                  75                  80
Ala Gly Glu Tyr Ala Thr Gly Ile Ala Phe Leu Pro Arg Gly Arg Met
                 85                  90                  95
Ala Met Met Asp Ala Gln Lys Glu Ile Glu Arg Ile Ala Lys Gln Glu
            100                 105                 110
Gly Ala Asp Val Leu Gly Trp Arg Met Val Pro Phe Asp Ser Arg Glu
        115                 120                 125
Leu Gly Ser Met Ala Glu Glu Ala Met Pro Ser Phe Ala Gln Ile Phe
    130                 135                 140
Leu Thr Val Pro Gly Lys Ser Gly Glu Asp Leu Asp Arg Val Met Phe
145                 150                 155                 160
Phe Ile Arg Lys Arg Cys Glu Arg Glu Leu Gly Thr Thr Asn Gly Arg
                165                 170                 175
Asp Thr Val Tyr Phe Pro Ser Leu Ser Ser Arg Thr Ile Ile Tyr Lys
            180                 185                 190
Gly Met Leu Thr Thr Leu Gln Leu Glu Gly Phe Phe Glu Asp Leu Gly
        195                 200                 205
Asp Ala Arg Leu Glu Ser Ala Ile Ala Ile Val His Ser Arg Phe Ser
    210                 215                 220
Thr Asn Thr Phe Pro Ser Trp Pro Leu Ala His Pro Tyr Arg Phe Val
225                 230                 235                 240
Ala His Asn Gly Glu Ile Asn Thr Val Arg Gly Asn Glu Asn Trp Met
                245                 250                 255
Arg Ala Arg Glu Ala Leu Ile Lys Asn Asp Lys Leu Gly Asn Leu Ser
            260                 265                 270
Ser Val Leu Pro Ile Cys Thr Pro Glu Gly Ser Asp Thr Ala Arg Phe
        275                 280                 285
Asp Glu Ala Leu Glu Leu Leu His Leu Gly Gly Tyr Ser Leu Pro His
    290                 295                 300
Ala Val Ala Met Met Ile Pro Gln Ala Trp Glu His Asn Lys Thr Leu
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Ser Pro Glu Leu Arg Asp Phe Tyr Glu Tyr His Ser Cys Leu Met Glu
325 330 335
Pro Trp Asp Gly Pro Ala Ala Leu Ala Phe Thr Asp Gly Arg Phe Val
340 345 350
Gly Ala Val Leu Asp Arg Asn Gly Leu Arg Pro Gly Arg Ile Thr Ile
355 360 365
Thr Asp Ser Gly Leu Val Val Met Ala Ser Glu Ser Gly Val Leu Asp
370 375 380
Leu Arg Glu Glu Ser Val Val Lys Arg Thr Arg Val Gln Pro Gly Arg
385 390 395 400
Met Phe Leu Val Asp Thr Ala Glu Gly Arg Ile Val Glu Asp Glu Glu
405 410 415
Ile Lys Gln Lys Leu Ser Glu Ala Gln Pro Tyr Gly Glu Trp Ile Arg
420 425 430
Asp Asn Phe Val His Leu Asp Arg Leu Pro Gln Thr Arg Tyr Asn Tyr
435 440 445
Met Ala His Ser Arg Ala Val Leu Arg Gln Arg Val Phe Gly Ile Thr
450 455 460
Glu Glu Asp Val Asp Leu Leu Leu Leu Pro Met Ala Arg Gln Gly Ala
465 470 475 480
Glu Ala Ile Gly Ser Met Gly Ser Asp Thr Pro Ile Ala Ala Leu Ser
485 490 495
Gln Arg Pro Arg Met Leu Tyr Asp Phe Phe Ala Gln Arg Phe Ala Gln
500 505 510
Val Thr Asn Pro Pro Leu Asp Ser Ile Arg Glu Lys Pro Val Thr Ser
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Met Phe Thr Leu Leu Gly Ala Gln Ser Asp Val Leu Asn Pro Gly Pro
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Asp Ala Ala Arg Arg Ile Arg Leu Glu Ser Pro Ile Ile Asp Asn His
545 550 555 560
Glu Leu Ala Thr Leu Ile Asn Ala Asn Ala His Gly Glu Trp Asp Ser
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Phe Gly Ala Ala Val Ile Ser Gly Leu Tyr Pro Val Ala His His Gly
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Ala Gly Met Lys Ala Ala Ile Ala Arg Val Arg Arg Glu Val Ser Glu
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Asp Glu Arg Met Ala Pro Ile Pro Ala Leu Leu Leu Thr Ser Ala Val
625 630 635 640
His Gln Tyr Leu Val Gln Gln Arg Thr Arg Thr Gln Cys Ser Leu Val
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Val Glu Ser Gly Asp Ala Arg Glu Val His His Leu Ala Met Leu Ile
660 665 670

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Gly Phe Gly Ala Asp Ala Ile Asn Pro Tyr Met Ala Phe Glu Thr Ile
        675                 680                 685
Asp Glu Leu Arg Met Lys Gly Gln Leu Gly Asp Leu Ser Leu Asp Glu
    690                 695                 700
Ala Ser Arg Asn Tyr Ile Lys Ala Ala Thr Thr Gly Val Leu Lys Val
705                 710                 715                 720
Met Ser Lys Met Gly Ile Ala Thr Val Ser Ser Tyr Arg Gly Ala Gln
                725                 730                 735
Leu Ala Asp Val Thr Gly Leu His Gln Asp Leu Leu Asp Asn Tyr Phe
            740                 745                 750
Gly Gly Ile Ala Ser Pro Ile Ser Gly Ile Gly Leu Asp Glu Val Ala
        755                 760                 765
Ala Asp Val Glu Ala Arg His Arg Ser Ala Phe Leu Pro Arg Pro Glu
    770                 775                 780
Glu His Ala His Arg Glu Leu Asp Leu Gly Gly Glu Tyr Lys Trp Arg
785                 790                 795                 800
Arg Glu Gly Glu Tyr His Leu Phe Asn Pro Glu Thr Ile Phe Lys Leu
                805                 810                 815
Gln His Ala Thr Arg Ser Gly Ser Tyr Glu Ile Phe Lys Asp Tyr Thr
            820                 825                 830
Arg Lys Val Asp Asp Gln Ser Thr Arg Leu Gly Thr Ile Arg Gly Leu
        835                 840                 845
Phe Glu Phe Ser Thr Asp Arg Lys Pro Ile Ser Val Ser Glu Val Glu
    850                 855                 860
Pro Val Ser Glu Ile Val Lys Arg Phe Ser Thr Gly Ala Met Ser Tyr
865                 870                 875                 880
Gly Ser Ile Ser Ala Glu Ala His Glu Val Leu Ala Ile Ala Met Asn
                885                 890                 895
Arg Leu Gly Gly Met Ser Asn Ser Gly Glu Gly Gly Glu Asp Ala Arg
            900                 905                 910
Arg Phe Asp Val Glu Pro Asn Gly Asp Trp Lys Arg Ser Ala Ile Lys
        915                 920                 925
Gln Val Ala Ser Gly Arg Phe Gly Val Thr Ser His Tyr Leu Asn Asn
    930                 935                 940
Cys Thr Asp Ile Gln Ile Lys Met Ala Gln Gly Ala Lys Pro Gly Glu
945                 950                 955                 960
Gly Gly Gln Leu Pro Pro Asn Lys Val Tyr Pro Trp Val Ala Glu Val
                965                 970                 975
Arg Ile Thr Thr Pro Gly Val Gly Leu Ile Ser Pro Pro Pro His His
            980                 985                 990
Asp Ile Tyr Ser Ile Glu Asp Leu Ala Gln Leu Ile His Asp Leu Lys
        995                1000                1005
Asn Ala Asn Pro Arg Ala Arg Ile His Val Lys Leu Val Ala Glu Gln
   1010                1015                1020
Gly Val Gly Thr Val Ala Ala Gly Val Ser Lys Ala His Ala Asp Val
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Val Leu Ile Ser Gly His Asp Gly Gly Thr Gly Ala Ser Pro Leu Thr
1045 1050 1055
Ser Leu Lys His Ala Gly Gly Pro Trp Glu Leu Gly Leu Ala Glu Thr
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Gln Gln Thr Leu Leu Leu Asn Gly Leu Arg Asp Arg Ile Arg Val Gln
1075 1080 1085
Cys Asp Gly Gln Leu Lys Thr Gly Arg Asp Val Val Ile Ala Ala Leu
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Leu Gly Ala Glu Glu Phe Gly Phe Ala Thr Ala Pro Leu Val Val Glu
105 1110 1115 1120
Gly Cys Ile Met Met Arg Val Cys His Leu Asp Thr Cys Pro Val Gly
1125 1130 1135
Ile Ala Thr Gln Asn Pro Asp Leu Arg Ser Lys Phe Thr Gly Lys Ala
1140 1145 1150
Glu His Val Val Asn Phe Phe Thr Phe Ile Ala Gln Glu Val Arg Glu
1155 1160 1165
Tyr Leu Ala Gln Leu Gly Phe Arg Ser Ile Asp Glu Ala Val Gly Gln
1170 1175 1180
Ala Gln Val Leu Arg Lys Arg Ser Gly Ile Pro Ala Asp Ser Arg Ala
185 1190 1195 1200
Ala His Leu Asp Leu Ser Pro Ile Phe His Arg Pro Glu Thr Pro His
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Phe Pro Thr Gln Asp Val Arg Cys Thr Lys Thr Gln Glu His Ser Leu
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Glu Lys Ala Leu Asp Asn Ala Phe Ile Asp Lys Ala Ser Asp Thr Ile
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Ile Ser Asn Val Asn Arg Ser Val Gly Thr Met Leu Gly Ser Ala Val
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Ser Arg Val Ala Gly Ala Gln Gly Leu Pro Asp Gly Thr Ile Thr Leu
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Asn Leu Gln Gly Cys Ala Gly Asn Ser Phe Gly Ala Phe Ile Pro Arg
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Gly Ile Thr Ile Asn Leu Thr Gly Asp Ala Asn Asp Phe Val Gly Lys
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Gly Leu Ser Gly Gly Lys Ile Val Ile Lys Pro Ser Ala Gln Ala Pro
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Lys Gln Leu Lys Asn Asn Pro Asn Ile Ile Ala Gly Asn Val Leu Gly
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Tyr Gly Ala Thr Ser Gly Glu Leu Phe Ile Arg Gly Gln Val Gly Glu
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Arg Phe Cys Val Arg Asn Ser Gly Ala Thr Ala Val Val Glu Gly Ile
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Gly Asn His Gly Cys Glu Tyr Met Thr Gly Gly Arg Val Leu Val Leu
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Gly Pro Val Gly Glu Asn Phe Gly Ala Gly Met Ser Gly Gly Ile Ala
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Tyr Leu Ala Asn Ser Pro Asp Leu Asn Gln Lys Ile Asn Gly Glu Leu
425                1430                1435                1440
Val Asp Val Val Pro Leu Ser Ala Asp Asp Leu Thr Trp Ala Asp Glu
               1445                1450                1455
Leu Ile Ala Arg His Arg Glu Leu Thr Gly Ser Glu Thr Lys Leu Arg
           1460                1465                1470
Ala Gln Asp Leu Val Lys Ile Met Pro Arg Asp Phe Gln Lys Val Leu
       1475                1480                1485
Asn Ile Ile Glu Thr Ala His Ala Glu Gly Gln Asp Pro Ala Ile Lys
   1490                1495                1500
Ile Met Glu Ala Val Ser
505                1510
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<210> 9
<211> 506
<212> PRT
<213> Brevibacterium lactofermentum

<400> 9

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Met Ala Asp Pro Gln Gly Phe Ile Lys Tyr Ser Arg Arg Glu Pro Ala
  1               5                  10                  15
His Arg Pro Val Pro Leu Arg Leu Met Asp Tyr Ser Glu Val Tyr Glu
             20                  25                  30
Lys Ala Pro Ala Gly Gln Ile Glu Glu Gln Ala Ala Arg Cys Met Asp
         35                  40                  45
Cys Gly Val Pro Phe Cys His Glu Gly Cys Pro Leu Gly Asn Ile Ile
     50                  55                  60
Pro Glu Trp Asn Asp Leu Val Arg Gln Gly Arg Trp Lys Glu Ala Tyr
 65                  70                  75                  80
Asp Arg Leu His Ala Thr Asn Asn Phe Pro Glu Phe Thr Gly Arg Leu
                 85                  90                  95
Cys Pro Ala Pro Cys Glu Gly Ala Cys Val Leu Gly Ile Asn Asp Asp
            100                 105                 110
Ser Val Thr Ile Lys Asn Val Glu Leu Glu Ile Val Glu Lys Ala Phe
        115                 120                 125
Arg Glu Gly Trp Val Gln Pro Val Val Ala Ser Met Ser Thr Gly Leu
    130                 135                 140
Ser Val Ala Val Val Gly Ser Gly Pro Ala Gly Leu Ala Ala Ala Gln
145                 150                 155                 160
Gln Leu Thr Arg Ala Gly His Ser Val Thr Val Phe Glu Arg Asp Asp
                165                 170                 175
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Arg Leu Gly Gly Leu Met Arg Tyr Gly Val Pro Glu Tyr Lys Met Glu
            180                 185                 190
Asn Arg Trp Ile Asp Arg Arg Ile Glu Gln Met Glu Ala Glu Gly Thr
        195                 200                 205
Thr Phe Gln Val Gly Thr Ser Pro Arg Ala Ala Glu Leu Ala Leu Phe
    210                 215                 220
Asp Ala Ile Leu Leu Ala Thr Gly Thr Pro Val Ala Arg Glu Leu Ser
225                 230                 235                 240
Val Pro Gly His Asp Leu Asn Gly Ile His Ala Ala Met Asp Tyr Leu
                245                 250                 255
Thr Ala Gln Asn Arg Ile Asn Glu Gly Asp Gly Glu Val Ser Pro Ile
            260                 265                 270
Asn Ala Lys Gly Lys Lys Val Val Ile Ile Gly Gly Gly Asp Thr Gly
        275                 280                 285
Thr Asp Cys Phe Gly Thr Ala Leu Arg Gln Gly Ala Glu Ser Val Thr
    290                 295                 300
Gln Phe Asp Ile Arg Pro Arg Ala Pro Phe Gln Arg Ala Asp Ser Thr
305                 310                 315                 320
Pro Trp Pro Met Tyr Pro Asn Leu Phe Arg Thr Ala Thr Ala His Glu
                325                 330                 335
Glu Gly Glu Tyr Ile Ile Thr Gly Asp Glu Ser Ala Asp Glu Ile Ala
            340                 345                 350
Ala Leu Gly Leu Ala Glu Arg Ala Ala Gly Ser Thr Leu Gly Glu Arg
        355                 360                 365
Lys Phe Ala Val Asn Thr Val Glu Phe His Gly Asn Asn Gly His Val
    370                 375                 380
Thr Gly Leu Thr Gly Asn Gln Ile Arg Val Val Asn Gly Lys Arg Glu
385                 390                 395                 400
Pro Ile Glu Gly Thr Glu Phe Pro Phe Glu Ala Asp Leu Val Leu Val
                405                 410                 415
Ala Leu Gly Phe Thr Gly Ala Glu Gln Gly Gly Leu Ala His Glu Leu
            420                 425                 430
Gly Val Gly Phe Asp Asp Arg Gly Arg Ile Leu Arg Asp Ser Glu Tyr
        435                 440                 445
Arg Ser Pro Thr Asn Ser Arg Val Tyr Ile Ala Gly Asp Asn Gly Arg
    450                 455                 460
Gly Gln Ser Leu Ile Val Trp Ala Ile Ala Glu Gly Arg Ala Cys Ala
465                 470                 475                 480
Ala Ala Ile Asp Ala Asp Leu Met Gly Glu Thr Ala Leu Pro Val Ala
                485                 490                 495
Val Ala Pro Gln Asp Val Pro Leu Ala Val
            500                 505
```

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A bacterial strain belonging to the genus *Corynebacterium* which has an ability to produce L-glutamic acid, wherein glutamine-oxoglutarate amino transferase activity is enhanced in a cell of said bacterial strain.

2. A strain according to claim 1, wherein the enhancement of the glutamine-oxoglutarate amino transferase activity is caused through amplifying the copy number of a gene encoding glutamine-oxoglutarate amino transferase.

3. A strain according to claim 1, wherein the enhancement of the glutamine-oxoglutarate amino transferase activity is caused through alteration of expression regulation sequence of a gene encoding glutamine-oxoglutarate amino transferase.

4. A strain according to any one of claims 1 to 3, wherein said glutamine-oxoglutarate amino transferase is derived from bacterium belonging to the genus *Escherichia* or *Corynebacterium*.

5. A strain according to claim 2 or 3, wherein said gene encoding glutamine-oxoglutarate amino transferase comprises a nucleotide sequence corresponding to at least nucleotide numbers of 565 to 6614 of a nucleotide sequence depicted in SEQ ID NO: 7.

6. A gene encoding glutamine-oxoglutarate amino transferase which comprises a nucleotide sequence corresponding to at least nucleotide numbers of 565 to 6614 of a nucleotide sequence depicted in SEQ ID NO: 7.

7. A method for producing L-glutamic acid by fermentation, comprising the steps of:

cultivating, in a liquid medium, a bacterial strain belonging to the genus Corynebacterium which has an ability to produce L-glutamic acid, wherein glutamine-oxoglutarate amino transferase activity is enhanced in a cell of said bacterial strain,

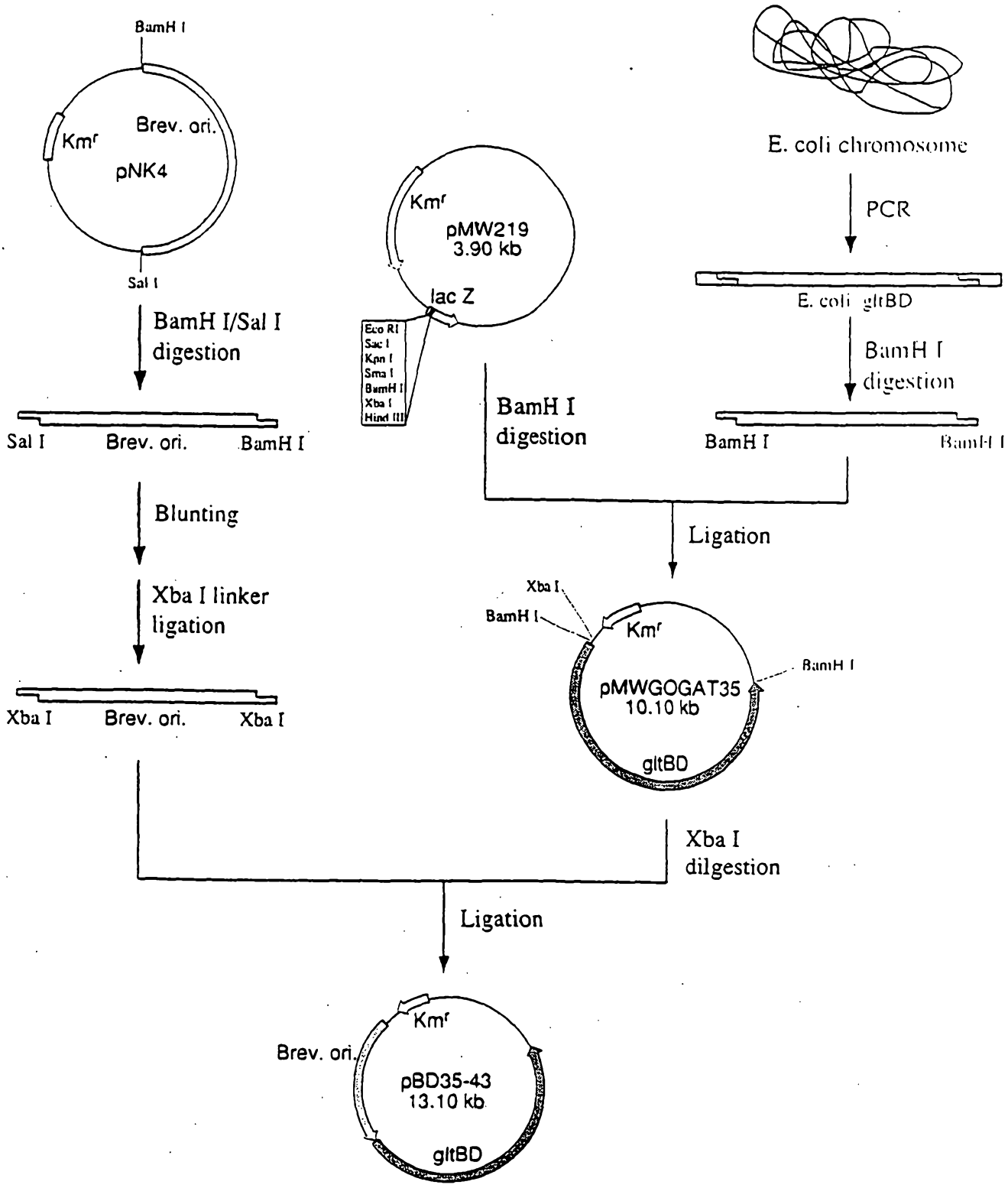
producing and accumulating L-glutamic acid in the culture medium, and

recovering the L-glutamic acid from the medium.

8. A bacterial strain according to claim 1, substantially as herein described with reference to any one of the examples or figures.

9. A method according to claim 7, substantially as herein described with reference to any one of the examples or figures.

Dated this 29th day of October 2001
AJINOMOTO CO., INC.
By their Patent Attorneys
GRIFFITH HACK
Fellows Institute of Patent and
Trade Mark Attorneys of Australia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Brev. ori.
pNK4
Sal I
BamH I/Sal I
digestion
Sal I
Brev. ori.
BamH I
Blunting
Xba I linker
ligation
Xba I
Brev. ori.
Xba I
Km^r
pMW219
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lac Z
Eco RI
Sac I
Kpn I
Sma I
BamH I
Xba I
Hind III
BamH I
digestion
E. coli chromosome
PCR
E. coli gltBD
BamH I
digestion
BamH I
BamH I
Ligation
Xba I
BamH I
Km^r
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gltBD
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Fig. 1