Patent Right	Date	September 24, 2020	Court	Tokyo District Court, 29th Civil Division
	Case number	2016(Wa)25436		
- A case in which a claim seeking an injunction and a claim for compensation for damages based on the patent right for an invention titled "Process for constructing amino acid-producing bacterium and process for producing amino acid by the fermentation method using the constructed amino acid-producing bacterium" and the patent right for an invention titled "L-glutamic acid-producing bacterium and process for producing L-glutamic acid" were partially affirmed.				

Summary of the Judgment

In this case, the Plaintiff holds the patent right of Patent No. 3651002 for an invention titled "Process for constructing amino acid-producing bacterium and process for producing amino acid by the fermentation method using the constructed amino acid-producing bacterium" (hereinafter referred to as "Patent Right 1") and the patent right of Patent No. 5343303 for an invention titled "L-glutamic acid-producing bacterium and process for producing L-glutamic acid" (hereinafter referred to as "Patent Right 2"). The Plaintiff alleged that the processes for producing the monosodium glutamate imported and sold by the Defendant (hereinafter the relevant monosodium glutamate is respectively referred to as "Defendant's Products 1 through 4" and the relevant processes are respectively referred to as "Defendant's Production Processes 1 through 4") fall within the technical scope of the invention claimed in Patent 1 or 2 (hereinafter respectively referred to as "Invention 1 or 2"), and that the Defendant's act of importing, transferring, and offering to transfer the Defendant's Products produced by the Defendant's Production Processes, by itself or jointly with its foreign affiliated company, constitutes infringement of Patent Right 1 with regard to Defendant's Products 1 and 3, and Patent Right 2 with regard to Defendant's Products 1 through 4. Based on this allegation, the Plaintiff made a claim seeking an injunction against transfer, import, and offer to transfer of the Defendant's Products, a claim seeking disposal of the Defendant's Products, and a claim for compensation for damages in tort due to patent infringement (a claim for damages under Article 102, paragraph (2) or (3) of the Patent Act and attorneys' fees).

The major issues of this case are as follows: [i] whether Defendant's Production Processes fall within the technical scope of Invention 1 or 2 (literal infringement / infringement under the doctrine of equivalents); [ii] whether there are grounds for

invalidation of the respective Patents (with regard to Patent 1, lack of an inventive step, violation of the enablement requirement, or violation of the support requirement; with regard to Patent 2, lack of novelty, lack of an inventive step, violation of the enablement requirement, violation of the support requirement, or violation of the clarity requirement); [iii] whether re-defense of correction of the respective Patents can be established; [iv] occurrence or non-occurrence and the amount of damages; and [v] whether or not the claim seeking an injunction and the claim seeking disposal are appropriate.

In this judgment, [i] the court held that Defendant's Production Processes 1 and 3 literally fall within the technical scope of Invention 1, and Defendant's Production Processes 1 through 3 fall within that of Invention 2, and that Defendant's Production Process 4, which has differences with the configuration specified in Invention 2, such as a difference in the species of bacterium from which the introduced mutant-type gene is derived, also falls within the technical scope of Invention 2 as an equivalent to the configuration specified in Invention 2. Although [ii] the court found that Invention 1 has a ground for invalidation, which is violation of the support requirement, [iii] it affirmed establishment of re-defense of correction except for some parts due to reasons such as that this ground for invalidation was eliminated by correction and no other grounds for invalidation are found at least in corrected Invention 1. The court also found establishment of re-defense of correction for Invention 2 on the basis that no grounds for invalidation are found at least in the corrected invention.

In this judgment, [iv] the court found the Defendant's act of importing, transferring, and offering to transfer as constituting the working of the Inventions for which the Defendant is liable for tort with regard to the Defendant's Products sold by the Defendant by itself, while finding the Defendant's act of offering to transfer in Japan as constituting the same with regard to the Defendant's Products relating to the portion sold by the affiliated company, and found establishment of joint tort by the Defendant and the affiliated company with regard to these acts of working the Inventions. In calculating the amount of damages under Article 102, paragraph (2) of the Patent Act relating to these acts, the court found that the presumption has been rebutted for a part of the marginal profit based on sale of competitive products, but holding that the amount of damages that takes the rebuttal into consideration exceeds the amount of the partial claim made by the Plaintiff in this case, the court entirely affirmed the amount claimed by the Plaintiff (the amount of the partial claim). [v] With regard to the claim seeking an injunction and a claim seeking disposal, the court denied the claims based on Patent Right 1 due to expiration of the patent term, and partially affirmed the claim seeking an

injunction and the claim seeking disposal based on Patent Right 2.

Judgment rendered on September 24, 2020 Original received on the same date by the court clerk

2016 (Wa) 25436 Appeal case of seeking injunction against patent right infringement

Date of conclusion of oral argument: March 19, 2020

Judgment

Indication of the parties: As stated in Attachment 1 "List of Parties."

Main text

1. The Defendant shall not transfer, import, or offer to transfer (including displaying for the purpose of transfer) the respective products specified in 1. and 2. of Attachment 17 "List of Products Subject to Injunction."

2. The Defendant shall dispose of the respective products specified in the preceding paragraph.

3. The Defendant shall pay to the Plaintiff 990 million yen and an amount accrued thereon at the rate of 5% per annum for the period from August 10, 2016, until the completion of the payment.

4. The Plaintiff's other claims shall be dismissed.

5. The court costs shall be divided into 20, and the Plaintiff shall bear one-twentieth thereof and the Defendant shall bear the remaining amount.

6. This judgment may be provisionally executed as far as paragraphs (1) and (3) are concerned.

Facts and reasons

[Table of Contents]

No. 1 Claims

1. The Defendant shall not transfer, import, or offer to transfer (including displaying for the purpose of transfer) the monosodium glutamate specified in 1. through 4. of Attachment 2 "List of the Defendant's Products."

2. The Defendant shall dispose of the monosodium glutamate specified in 1. through 4. of Attachment 2 "List of the Defendant's Products" which are under the Defendant's

possession.

3. The same as paragraph (3) of the main text.

No. 2 Outline of the case

1. Summary of the case

(1) Outline of the Plaintiff's claims

In this case, the Plaintiff holds the patent right of Patent No. 3651002 for an invention titled "Process for constructing amino acid-producing bacterium and process for producing amino acid by the fermentation method using the constructed amino acid-producing bacterium" (hereinafter, this patent right is referred to as "Patent Right 1" and this patent is referred to as "Patent 1") and the patent right of Patent No. 5343303 for an invention titled "L-glutamic acid-producing bacterium and process for producing L-glutamic acid" (hereinafter, this patent right is referred to as "Patent Right 2" and this patent is referred to as "Patent 2"; in addition, Patent Right 1 and Patent Right 2 are collectively referred to as the "Patent Rights" and Patent 1 and Patent 2 are collectively referred to as the "Patents"). The Plaintiff alleged that the processes for producing the monosodium glutamate specified in 1. through 4. of Attachment 2 "List of the Defendant's Products" (hereinafter, each monosodium glutamate is referred to as "Defendant's Product 1" or the like according to the number assigned to it on that list, and they are collectively referred to as the "Defendant's Products") respectively by the corresponding processes specified in Attachment 3 "List of the Defendant's Production Processes" (hereinafter, each process is referred to as "Defendant's Production Process 1" or the like according to the number assigned to it on that list, and they are collectively referred to as the "Defendant's Production Processes") fall within the technical scopes of the inventions claimed in Patents 1 and 2, and that the Defendant's act of importing, transferring, and offering to transfer the Defendant's Products produced by the Defendant's Production Processes, by itself or jointly with its affiliated company, constitutes infringement of Patent Right 1 with regard to Defendant's Products 1 and 3, as well as Patent Right 2 with regard to Defendant's Products 1 through 4. Based on this allegation, the Plaintiff made the following claims.

A. Under Article 100, paragraph (1) of the Patent Act, a claim seeking an injunction against transfer, import, and offer to transfer (including displaying for the purpose of transfer) of the Defendant's Products (paragraph (1) of the object of claims)

B. Under Article 100, paragraph (2) of the Patent Act, a claim seeking disposal of the Defendant's Products which are under the Defendant's possession (paragraph (2) of the object of claims)

C. Based on the right to claim compensation for damages in tort due to patent

infringement, a claim for payment of damages in the amount of 990 million yen (a total of 900 million yen as partial claims for the damages under Article 102, paragraph (2) or (3) of the Patent Act and 90 million yen as attorneys' fees), with delay damages accrued thereon at the rate of 5% per annum as prescribed in the Civil Code (the Code prior to the amendment by Act No. 44 of 2017; the same applies hereinafter) for the period from August 10, 2016 (the day following the date of service of the complaint), which is a day after the act of tort, until the completion of the payment (paragraph (3) of the object of claims)

The period subject to the claim for compensation for damages is the period from January 1, 2007, to December 31, 2017, for the infringement of Patent Right 1 (hereinafter referred to as the "subject period") and the period from August 23, 2013, which is the registration date of Patent Right 2, to December 31, 2017, for the infringement of Patent Right 2 (hereinafter, this period may be referred to as the "subject period for Patent Right 2"), and the abovementioned amount was selectively claimed as partial claims of the claims for compensation for damages due to the respective patent infringements in these periods.

(2) Outline of the Defendant's allegations

The Defendant, while recognizing that Defendant's Production Process 1 was being used in a part of the subject period, disputes the Plaintiff's allegations with regard to the use or non-use, the periods of use, etc. of the Defendant's Production Processes. In addition, in regard to whether the Defendant's Production Processes fall within the technical scopes of the inventions claimed in the Patents, the Defendant disputes the existence or non-existence of infringement of Patent Right 2 under the doctrine of equivalents mainly regarding Defendant's Product 4. Moreover, the Defendant alleges that both Patent 1 and Patent 2 had grounds for invalidation before and after the corrections, and hence the Defendant's Production Processes have not infringed the Patent Rights.

Furthermore, the Defendant disputes the necessity of an injunction on the basis that the Defendant's Products are currently not produced. Also, with regard to the existence or non-existence and the scope of acts of working the inventions claimed in the Patents for which the Defendant should be held liable for compensation for damages in connection with the claim for compensation for damages caused by acts conducted during the subject period, the Defendant alleges that, whereas the Defendant's Products were produced in the Republic of Indonesia (hereinafter referred to as "Indonesia"), the Defendant's Products exported to Japan include a portion directly sold to Japanese customers by the Indonesian corporation which produced the products, and argues that this portion does

not constitute working of the inventions in Japan because the act of sale was conducted outside Japan, and that the Defendant should not be held liable for compensation for damages with regard to this portion. Thus, the Defendant disputes the Plaintiff's method of calculation of damages.

2. Basic facts, etc. (facts not disputed between the parties or facts that can easily be found based on the evidence mentioned below (hereinafter, any branch numbers of documentary evidence are omitted unless otherwise indicated) and the entire import of oral arguments, etc.)

(1) Parties, etc.

A. The Plaintiff

The Plaintiff is a company engaged in the production, processing, purchase and sale, import and export, research and development, etc. of products of seasonings, sweeteners, and various amino acids, as well as their ingredients, byproducts, and related goods in the course of trade, and produces monosodium glutamate (MSG) products named "Ajinomoto" (the entire import of oral arguments).

B. The Defendant and the corporate group to which the Defendant belongs

(A) The Defendant is a Japanese corporation engaged in the processing and sale of seasonings and spices including monosodium glutamate and nucleic acids, processing and sale of feeds and feed additives, sale of seasonings and food additives applying fermentation chemistry, organic chemistry, and inorganic chemistry, etc. in the course of trade (the entire import of oral arguments).

(B) The Defendant belongs to a corporate group centered on a South Korean corporation, CJ CheilJedang Corporation (hereinafter, the corporation is referred to as "CJCJ" and the group is referred to as the "CJ Group"). In the CJ Group, the group companies share roles in producing and selling CJ-brand and other products in countries around the world.

The Defendant is a wholly owned subsidiary company of a South Korean corporation "CJ CORP." (hereinafter referred to as "CJ Company"), which is the holding company, and CJ Company holds 44% of CJCJ's shares (Exhibit Otsu 108; the entire import of oral arguments).

(C) "PT CHEIL JEDANG INDONESIA" (hereinafter referred to as "CJ Indonesia") is an Indonesian corporation which belongs to the CJ Group and is a wholly owned subsidiary company of CJCJ (Exhibit Otsu 108; the entire import of oral arguments; hereinafter, the Defendant and CJ Indonesia may be collectively referred to as the "Defendant, etc.").

(2) Production of monosodium glutamate by the fermentation method

One process for producing glutamic acid and other amino acids is the amino acid fermentation method (hereinafter referred to as the "fermentation method") whereby

ingredients such as molasses are added to a medium for culturing microorganisms to cause the microorganisms to produce amino acids as they propagate.

Well-known glutamic acid-producing bacteria used for the fermentation method include bacteria of the genus *Corynebacterium*, which are coryneform bacteria.

One of the general production processes of monosodium glutamate using the fermentation method is as follows: glutamic acid-producing bacteria are placed in a fermentation vessel (fermentation tank) containing major ingredients, such as the carbon source represented by molasses, and are propagated; L-glutamic acid (hereinafter, "L-glutamic acid" is simply referred to as "glutamic acid" unless otherwise indicated) is produced by having the carbon source (sugar) converted into glutamic acid through metabolic reactions within the bacterial cell; after the end of this fermentation process, crystals are precipitated from the fermented liquid and glutamic acid crystals are collected by a separation operation; the collected glutamic acid is neutralized by caustic soda and then decolorized with activated carbon; and monosodium glutamate crystals are obtained (Exhibits Ko 8 and 9, Exhibit Otsu 2).

The Patent Rights relate to processes for producing glutamic acid by the fermentation method.

(3) Patent Rights

The Plaintiff is the patentee of the Patent Rights, and the filing dates, etc. of the Patent Rights are as shown below (Exhibits Ko 1 through 4). The term of Patent Right 1 expired on September 22, 2019.

A. Patent Right 1

Patent number: Patent No. 3651002

Filing date: July 8, 2004

Application number: Patent Application No. 2004-202121

Registration date: March 4, 2005

Filing date of the original application: September 22, 1999

Priority date: September 25, 1998 (hereinafter referred to as "Priority Date 1")

Priority claim country: Japan

Title of the invention: Process for constructing amino acid-producing bacterium and process for producing amino acid by the fermentation method using the constructed amino acid-producing bacterium

B. Patent Right 2

Patent number: Patent No. 5343303

Filing date: December 28, 2005

Application number: Patent Application No. 2005-379259

Registration date: August 23, 2013
Priority date: December 28, 2004 (hereinafter referred to as "Priority Date 2")
Priority claim country: Japan
Title of the invention: L-glutamic acid-producing bacterium and process for producing L-glutamic acid

(4) Claims, etc. of the invention claimed in Patent 1

A. Claims before corrections

Claims 1 through 4 of the claims of Patent 1 are as shown in Attachment 4-1 "Claims (Patent 1)."

The inventions claimed in Claims 1 through 4 (hereinafter, these inventions are referred to as "Invention 1-1," "Invention 1-2," "Invention 1-3," and "Invention 1-4" in the respective order, and collectively as "Invention 1") can be divided into constituent features as shown in Attachment 4-3 "Division into Constituent Features (Invention 1)" (hereinafter, each divided constituent feature is referred to as "Constituent Feature 1-A - 1" or the like according to the code assigned at the beginning of each constituent feature; the same applies to the division of claims in Attachments 4-4, 5-4, 5-5, and 5-6).

B. Requests for correction

On July 7, 2017, the Plaintiff filed a request for correction of Patent 1 (hereinafter referred to as "Correction 1") in the proceedings of a trial for patent invalidation filed by the Defendant (Invalidation Trial No. 2017-800021) (Exhibit Ko 93, Exhibit Otsu 69).

Claims 1, 2, and 4 (Claim 3 was deleted) of the claims corrected by Correction 1 are as shown in Attachment 4-2 "Corrected Claims (Patent 1)."

The inventions claimed in Claims 1, 2, and 4 after the correction (hereinafter, these inventions are referred to as "Corrected Invention 1-1" (Claim 1), "Corrected Invention 1-2" (Claim 2), and "Corrected Invention 1-3" (Claim 4) in the respective order, and collectively referred to as "Corrected Invention 1") can be divided into constituent features as shown in Attachment 4-4 "Division into Constituent Features (Corrected Invention 1)."

As the corrections in Correction 1 are all made within the scope of the matters stated in the description, claims, or drawings attached to the written application, for the purpose of restricting the claims or clarifying an ambiguous statement (items (i) and (iii) of Article 134-2, paragraph (1) of the Patent Act), and do not substantially enlarge or alter the claims, they satisfy the requirements for correction (Article 134-2, paragraph (9) and Article 126, paragraphs (5) and (6) of the Patent Act) (Exhibits Ko 50-1 and 93; the entire import of oral arguments).

C. JPO decision, etc.

With regard to the request for a trial for patent invalidation referred to in B. above, the JPO rendered a trial decision on January 8, 2019, affirming Correction 1, holding that the patent is to be maintained with regard to the portion other than that deleted by the correction (Exhibit Ko 93). In response, the Defendant filed a lawsuit seeking rescission of the JPO decision, and as of the time of conclusion of the oral argument of this case, the abovementioned JPO decision has yet to become final and binding (the entire import of oral arguments).

(5) Claims, etc. of the invention claimed in Patent 2

A. Claims before the corrections

Claims 1, 4, 6, 10, and 11 of the claims of Patent 2 are as shown in Attachment 5-1 "Claims (Patent 2)."

The inventions claimed in Claims 1, 4, 6, 10, and 11 (hereinafter, these inventions are referred to as "Invention 2-1" (Claim 1), "Invention 2-2" (Claim 4), "Invention 2-3" (Claim 6), "Invention 2-4" (Claim 10), and "Invention 2-5" (Claim 11) in the respective order, and collectively referred to as "Invention 2") can be divided into constituent features as shown in Attachment 5-4 "Division into Constituent Features (Invention 2)."

B. Request for correction

On July 7, 2017, the Plaintiff filed a request for correction of Patent 2 (hereinafter referred to as "Correction 2") in the proceedings of a trial for patent invalidation filed by the Defendant (Invalidation Trial No. 2017-800022) (Exhibit Ko 94, Exhibit Otsu 70).

Claims 1, 4, 6, 10, and 11 of the claims corrected by Correction 2 are as shown in Attachment 5-2 "Corrected Claims (Patent 2)."

The inventions claimed in Claims 1, 4, 6, 10, and 11 after the correction (hereinafter, these inventions are referred to as "Corrected Invention 2-1" (Claim 1), "Corrected Invention 2-2" (Claim 4), "Corrected Invention 2-3" (Claim 6), "Corrected Invention 2-4" (Claim 10), and "Corrected Invention 2-5" (Claim 11) in the respective order, and collectively referred to as "Corrected Invention 2") can be divided into constituent features as shown in Attachment 5-5 "Division into Constituent Features (Corrected Invention 2)."

C. JPO decision, etc.

With regard to the request for a trial for patent invalidation referred to in B. above, the JPO rendered a trial decision on January 8, 2019, affirming Correction 2, holding that the patent is to be maintained with regard to the portion other than that deleted by the correction (Exhibit Ko 94). In response, the Defendant filed a lawsuit seeking rescission of the JPO decision, and as of the time of conclusion of the oral argument of this case, the abovementioned JPO decision has yet to become final and binding (the entire import of

oral arguments).

D. Plan for further correction

As an alternative claim to the claim based on Correction 2 regarding Patent 2, the Plaintiff asserts that it plans to make further correction that differs from Correction 2 (hereinafter, that further correction may be referred to as "re-correction of Patent 2") when it becomes procedurally possible to do so, but as of the time of conclusion of the oral argument of this case, a request for such correction has not been filed (the entire import of oral arguments).

Claims 4, 13, and 14 of the claims to be corrected by re-correction of Patent 2 are as shown in Attachment 5-3 "Re-corrected Claims (Patent 2)" (Exhibit Ko 53).

The inventions claimed in Claims 4, 13, and 14 after the re-correction of Patent 2 (hereinafter, these inventions are referred to as "Re-corrected Invention 2-2" (Claim 4), "Re-corrected Invention 2-3" (Claim 13), and "Re-corrected Invention 2-6" (Claim 14) in the respective order, and collectively referred to as " Re-corrected Invention 2") can be divided into constituent features as shown in Attachment 5-6 "Division into Constituent Features (Re-corrected Invention 2)."

(6) Descriptions, etc. of the Patents

The description and drawings of Patent 1 (Exhibit Ko 3) are referred to as "Description 1," and the description and drawings of Patent 2 (Exhibit Ko 4) are referred to as "Description 2."

In addition, unless otherwise indicated, when describing working examples, paragraph numbers (to be described as [0001] or the like), or drawings or table numbers (to be described as [Table 1], [Figure 1], or the like) contained in the detailed explanation of the invention of the description with regard to the contents of the inventions claimed in the Patents, they respectively indicate the corresponding parts of Description 1 for the inventions claimed in Patent 1 and the corresponding parts of Description 2 for the inventions claimed in Patent 2. Meanwhile, as paragraph [0033] of Description 2 has been corrected by Correction 2, when citing that part, a clear indication is made as to whether the cited contents are those before or after the correction.

(7) Defendant's Products and Defendant's Production Processes

A. Import and sale of monosodium glutamate products by the Defendant

The Defendant imported monosodium glutamate with the product name "MI-POONG" (hereinafter referred to as the "MSG in question") into Japan from Indonesia, offered to transfer it and transferred it from 2007 to at least until the end of 2017.

The MSG in question was produced by the fermentation method by CJ Indonesia in a factory in Indonesia (Exhibit Otsu 2, the entire import of oral arguments).

The Defendant alleges that the MSG in question which was exported from Indonesia to Japan during the subject period and is distributed in Japan includes not only the portion imported and sold by the Defendant (hereinafter referred to as the "portion sold by the Defendant"), but also the portion directly sold by CJ Indonesia to Japanese customers (hereinafter referred to as the "portion sold by CJ Indonesia," and together with the portion sold by the Defendant, they are referred to as the "portion sold by the Defendant, etc."), and, as mentioned below, there is a dispute between the parties as to whether the Defendant is liable for tort due to infringement of the Patent Rights with regard to the portion sold by CJ Indonesia.

B. Scope, etc. of products subject to the Plaintiff's claims

Of the MSG in question, the scope of products specified by the Plaintiff as those subject to the Plaintiff's claims are the Defendant's Products described in Attachment 2 "List of the Defendant's Products" that were produced by the Defendant's Production Processes described in Attachment 3 "List of the Defendant's Production Processes."

With regard to the relationship between the Defendant's Production Processes and the inventions claimed in the Patents, the Plaintiff alleges as follows: both before and after the corrections, Defendant's Production Process 1 falls within the technical scope of the inventions claimed in Patents 1 and 2, Defendant's Production Process 2 falls within the technical scope of the inventions claimed in Patent 2, Defendant's Production Process 3 falls within the technical scope of the inventions claimed in Patents 1 and 2, and Defendant's Production Process 4 falls within the technical scope of the inventions claimed in Patent 2 as an equivalent thereto.

(8) Prior art documents

A. Regarding Patent 1

The following documents, etc. existed before September 25, 1998, which is Priority Date 1.

(A) *Microbiology* (1996) 142, p. 1297–1309 (Exhibit Otsu 6; hereinafter, the document is referred to as "Exhibit Otsu 6 Document" and the invention described in Exhibit Otsu 6 Document is referred to as "Exhibit Otsu 6 Invention"; similarly, unless otherwise indicated, the following documents are also respectively referred to as "Exhibit Otsu 6 Document" or the like according to the corresponding Exhibit Otsu number, and the inventions described in those documents are described as "Exhibit Otsu 6 Invention" or the like)

(B) *Microbiology* (1994) 140, p. 1817–1828 (Exhibit Otsu 8, Exhibit Ko 25)

(C) International Publication No. 95/34672 (Exhibit Otsu 9)

(D) *EMBO J.* (1982) 1 (7), p. 875–881 (Exhibit Otsu 10)

(E) *J. Biol. Chem.*(1985) 260 (6), p. 3539–3541 (Exhibit Otsu 11)
(F) Unexamined Patent Application Publication No. 1991–147792 (Exhibit Otsu 12)
(G) Unexamined Patent Application Publication No. 1988–214189 (Exhibit Otsu 35)
B. Regarding Patent 2

The following documents, etc. existed before December 28, 2004, which is Priority Date 2.

(A) *Eur. J. Biochem.* (1997) 247, p. 572–580 (Exhibit Otsu 24)
(B) *FEMS Microbiol. Lett.* (2003), 218, p. 305–309 (Exhibit Otsu 26)
(C) *Science* (2002) 298 (22), p. 1582–1587 (Exhibit Otsu 29)
(D) *EMBO J.* (2003) 22 (1), p. 36–46 (Exhibit Otsu 30)
(E) *Biophysical J.* (2004. Nov.) 87, p. 3050–3065 (Exhibit Otsu 31)
(F) International Publication No. 2003/046123 (Exhibit Otsu 41-1), U.S. Patent Application Publication No. 2005/0003494 Description (Exhibit Otsu 41-2; it is the U.S. patent application publication corresponding to the international application of Exhibit Otsu 41-1, and together with Exhibit Otsu 41-1 it is referred to as "Exhibit Otsu 41 Document")
(G) European Patent Application Publication No. 1108790 Description (Exhibit Otsu 42)
3. Issues
(1) Use or non-use, the periods of use, etc. of the Defendant's Production Processes (Issue 1)
(2) Whether the Defendant's Production Processes fall within the technical scopes of the inventions claimed in the Patents (Issue 2)
A. Whether Defendant's Production Processes 1 and 3 literally fall within the technical scope of Invention 1 (Issue 2-1)
B. Whether Defendant's Production Processes 1 through 3 literally fall within the technical scope of Invention 2 (Issue 2-2)
C. Whether Defendant's Production Process 4 falls within the technical scope of Invention 2 as an equivalent thereto (Issue 2-3)
(3) Whether Patent 1 should be invalidated by a trial for patent invalidation (Issue 3)
A. Whether or not Invention 1 lacks an inventive step based on Exhibit Otsu 6 Invention as the primary cited document (Issue 3-1)
B. Whether or not Invention 1 lacks an inventive step based on Exhibit Otsu 9 Invention as the primary cited document (Issue 3-2)
C. Whether or not Invention 1 violates the enablement requirement or the support requirement (Issue 3-3)
(4) Whether Patent 2 should be invalidated by a trial for patent invalidation (Issue 4)

A. Whether or not Invention 2 lacks novelty based on Exhibit Otsu 42 Invention (Issue 4-1)
B. Whether or not Invention 2 lacks an inventive step based on Exhibit Otsu 24 Invention as the primary cited document (Issue 4-2)
C. Whether or not Invention 2 violates the enablement requirement or the support requirement (Issue 4-3)
D. Whether or not Invention 2 violates the clarity requirement (Issue 4-4)
(5) Whether re-defense of correction of Patent 1 can be established (Issue 5)
(6) Whether re-defense of correction of Patent 2 (Correction 2) can be established (Issue 6)
(7) Whether re-defense of re-correction of Patent 2 can be established (alternative claim to the re-defense of Issue 6) (Issue 7)
(8) Occurrence or non-occurrence and the amount of damages (Issue 8)
(9) Whether or not the claim seeking injunction and the claim seeking disposal are appropriate (Issue 9)

(omitted)

No. 4 Judgment of this court
1. Regarding Issue 1 (use or non-use, the periods of use, etc. of the Defendant's Production Processes)
(1) Regarding the period from January 2011 through May 2014
A. There is no dispute between the parties concerning the fact that the MSG in question was being produced by Defendant's Production Process 1 during the period from January 2011 through May 2014, during which the Defendant alleges using Strain [ii].
Therefore, the strain being used for producing the MSG in question during this period was *Corynebacterium glutamicum*, in which genes on the chromosome have the characteristics described in 1. of Attachment 2 "List of the Defendant's Products" (the same characteristics as those described for Strain [ii] in the "Defendant's allegations" column of Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question").
B. Regarding analysis results of PROMATE2012
According to the evidence (Exhibits Ko 15 and 22) and the entire import of oral arguments, on November 30, 2012, which is a date included in the relevant period, the Plaintiff obtained and analyzed PROMATE produced in CJ Indonesia's factory (PROMATE is livestock feed made by utilizing used bacterial cells produced when

producing the MSG in question; hereinafter, this PROMATE obtained by the Plaintiff is referred to as "PROMATE2012"), and as a result, confirmed that the strain contained in PROMATE2012 had the characteristics referred to in A. above.

(2) Regarding the period from August 2015 through June 2016

A. With regard to this period in which the Defendant alleges using Strain [x], the Plaintiff alleges, based on the analysis results (Exhibit Ko 49) of the obtained PROMATE2015, that the same strain as that in the period referred to in (1) above was being used, whereas the Defendant alleges that a strain having different characteristics from that in (1) above was being used.

B. The Plaintiff's analysis results and the credibility thereof

(A) According to the evidence (Exhibits Ko 49, 66, and 67) and the entire import of oral arguments, it was found that, on December 30, 2015, the Plaintiff obtained and analyzed PROMATE2015 produced by CJ Indonesia, and that according to the analysis results (Exhibit Ko 49), the following mutations were detected in the DNA extracted from PROMATE2015, as in the case with those detected in PROMATE2012: a mutation that alters the -35 region of the promoter sequence of the GDH gene to TTGTCA and the -10 region to TATAAT; a mutation that alters the -10 region of the promoter sequence of the CS gene to TATAAT; and a mutation in which alanine at position 100 in the amino acid sequence encoded by the yggB gene is substituted with threonine.

(B) The Defendant disputes the credibility of the Plaintiff's analysis results (Exhibit Ko 49), alleging that it could not confirm those analysis results in the results of CJCJ's analysis of PROMATE which was produced on the same date as PROMATE2015 (Exhibit Otsu 75). However, CJCJ's analysis failed to conduct sequence analysis with regard to a part of the analysis of the promoter region of the GDH gene and the analysis of the promoter region of the CS gene and the yggB gene, due to reasons such as weak signals, and in spite of obtaining such results, there is no sign of CJCJ having conducted analysis, etc. again under different conditions. Therefore, it should be said that CJCJ's analysis lacks credibility as analysis conducted by a person skilled in the art of processes for producing the MSG in question (Exhibit Otsu 2), and that it is not sufficient to overturn the credibility of the Plaintiff's analysis results.

Consequently, it should be said that the Plaintiff's analysis results which indicated that the mutations referred to in (A) above, which are the same as those detected in the analysis of PROMATE2012, were detected in the DNA derived from PROMATE2015 are credible.

C. Regarding inclusion of a strain used in the past

According to the Plaintiff's analysis results (Exhibit Ko 49), it is found that, in the promoter sequence of the GDH gene of PROMATE2015, [i] sequences having the same

mutations as those in PROMATE2012, [ii] wild-type sequences, and [iii] sequences that differ from both of the former two were detected, and in regard to their percentages, [i] above accounted for the highest percentage whereas [ii] accounted for the lowest percentage, while the ratio of their signal intensities was 5:1:2 in the order of their numbers.

The Defendant alleges that, because powder made by crushing PROMATE that was used in the past is added as seed when producing PROMATE, and also because some residues remain within the production equipment, components derived from the past strain are present along with components derived from the most recently used strain.

According to the evidence (Exhibit Otsu 88) and the entire import of oral arguments, it is found that, at the stage of production of PROMATE, not only the strain that was being used for producing the MSG in question at the time was used as an ingredient, but also past PROMATE that had been produced earlier was added as seed for promoting granulation. However, while the Defendant explains that the percentage of seed added was typically 30%, the sequences having the same mutations as those in PROMATE2012 were detected from PROMATE2015 at the highest percentage, whereas the wild-type sequences, which the Defendant alleges to have been the sequences of the strain used at the time, were detected at the lowest percentage, as mentioned above, and hence, the sequences having the abovementioned mutations are unlikely to have been added as seed.

Although the Defendant also indicates the effect of residues within the production equipment, the fact that the sequences having the same mutations as those in PROMATE2012 were detected at the highest percentage as mentioned above is difficult to explain by the effect of residues.

In addition, the Defendant indicates that PROMATE is livestock feed, and it is not produced in a manner such that bacterial cells are distributed evenly among the particles, but as the Defendant has not shown analysis results that indicate different results from the Plaintiff's analysis results with regard to the percentage of sequences having the same mutations as those in PROMATE2012, it cannot be said that this sufficiently explains the reason for which the past strain is detected more strongly than the present strain.

In this way, even if the points indicated by the Defendant are taken into consideration, it is reasonable to find that the mutations referred to in B. (A) above detected from PROMATE2015 were derived from the strain that was being used for producing the MSG in question as of December 30, 2015, and there is no sufficient evidence to overturn this finding.

D. Consequently, it is reasonable to presume that the strain that was being used around December 30, 2015, when PROMATE2015 was produced, was a strain which has the

same characteristics as Strain [ii] and which was used in Defendant's Production Process 1, and that this strain was being used at least during the period from around August 2015 through around June 2016 in which the Defendant alleges using Strain [x].

As the Defendant admits that the MSG in question was produced by the fermentation method, and does not specifically dispute points other than the type of the strain with regard to the use or non-use of Defendant's Production Process 1 during the relevant period, it is found that the MSG in question produced during the relevant period was Defendant's Product 1 produced by Defendant's Production Process 1.

(3) Regarding other periods

A. The Plaintiff asserts that Defendant's Production Process 1 was being used not only in the periods referred to in (1) and (2) above, but also in the respective periods of Strain Unknown Period [i] and the periods in which the Defendant alleges using Strains [iii] through [ix] and Strains [xi] through [xiv], but there are no materials directly supporting the fact that Defendant's Production Process 1 was being used in these periods.

In addition, as mentioned in (2) C. above, when producing PROMATE, components derived from a strain that had been used for producing the MSG in question earlier may be mixed in, and according to the Plaintiff's analysis results of PROMATE2015, sequences other than the sequences having the same mutations as those in PROMATE2012 were also detected in the promoter sequence of the GDH gene. This is consistent with the Defendant's allegation that, when producing the MSG in question, the same strain was not always being used, but different strains were being used according to the period.

Regarding the characteristics of the genes of the strains that were being used, the Defendant makes allegations as shown in Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question," except for Strain Unknown Period [i], and the Defendant has disclosed analysis results that are consistent with those characteristics with regard to strains that were used in some periods (Exhibit Otsu 74). When considering these facts, it cannot be presumed based on the analysis results of PROMATE2012 and PROMATE2015, which were examined in (1) and (2) above, that Defendant's Production Process 1 was also being used in periods other than those referred to in (1) and (2) above, and there is no other evidence sufficient for affirming the Plaintiff's allegation regarding this point.

Accordingly, it is reasonable to find that *Corynebacterium glutamicum*, which has the characteristics described in the "Defendant's allegations" column of Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question," was being used in the respective periods of Strain Unknown Period [i] and in

the periods during which the Defendant alleges using Strains [iii] through [ix] and Strains [xi] through [xiv].

B. As the Defendant admits that the MSG in question was produced by the fermentation method, and does not specifically dispute points other than the type of the strain with regard to the use or non-use of the Defendant's Production Processes, it is reasonable to find that the state of use of the Defendant's Production Processes in production of the MSG in question during the periods of use of the respective strains that were examined in A. above was the same as that in the "Plaintiff's alternative claims" column of Attachment 9, and the Defendant's Production Processes are not found to have been used in Strain Unknown Period [i] and the period of use of Strain [xiv].

C. The Plaintiff asserts that it is unconceivable that the Defendant does not possess records of strains that were used only six years ago with regard to Strain Unknown Period [i], and that, as long as the Defendant cannot prove that it was using a bacterium that differs from Strain [ii], the Defendant should naturally be presumed to have been using the same bacterium as Strain [ii]. However, considering that the analysis results of PROMATE2015 also suggest that different strains were being used depending on the period for producing the MSG in question, as mentioned in A. above, it cannot naturally be presumed that the Defendant was using the same strain as Strain [ii] in Strain Unknown Period [i] only based on the fact that the Defendant has not alleged or proved the specific characteristics of the strain that it was using.

In addition, with regard to Strains [xii] and [xiii], the Plaintiff alleges that if the sequence of the yggB gene had been altered from earlier strains, as alleged by the Defendant, an application for the safety assessment required under the Food Sanitation Act should have been filed, but as the application has not been filed, the Defendant's allegation cannot be trusted. However, according to the Plaintiff's allegation, the Defendant should have filed an application for the abovementioned safety assessment also for the MSG produced by using strains having a mutant-type yggB gene with an A100T-type mutation (Strains [ii] through [xi]), but the Defendant failed to do so. Therefore, even if the Defendant did not file an application for the abovementioned safety assessment in the periods before and after the use of Strains [xii] and [xiii], it cannot be said that the same mutant-type yggB gene as earlier was being used for Strains [xii] and [xiii] based on the Defendant's failure to file the application, and this point also is insufficient for overturning the determination in B. above.

(4) Summary of the periods of use of the Defendant's Production Processes

According to (1) through (3) above, with regard to the use or non-use of the Defendant's Production Processes in the respective periods, it is found that Defendant's

Production Process 1 was being used during the period of use of Strains [ii] and [x] described in Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question" and, during other periods, the state of use was the same as that described in the "Plaintiff's alternative claims" column of that Attachment.

2. Regarding Issue 2-1 (whether Defendant's Production Processes 1 and 3 literally fall within the technical scope of Invention 1)

According to the entire import of oral arguments, it is found that, when Defendant's Production Processes 1 and 3 are compared with Invention 1, the results of the comparison will be the same as the description in Attachment 6-1 "Comparison between Defendant's Production Processes 1 and 3 and Invention 1," and therefore Defendant's Production Process 1 literally falls within the technical scopes of Inventions 1-1 through 1-4, and Defendant's Production Process 3 literally falls within the technical scopes of Inventions 1-1 and 1-4.

3. Regarding Issue 2-2 (whether Defendant's Production Processes 1 through 3 literally fall within the technical scope of Invention 2)

(1) Regarding Defendant's Production Process 1

According to the analysis results of PROMATE2012 (Exhibit Ko 22), it is found that, with regard to Strain [ii], an A100T mutation is introduced into the amino acid sequence of SEQ ID NO: 6 of the yggB gene, and the amino acid sequence of the yggB gene after the mutation coincides with the amino acid sequence of SEQ ID NO: 22. In addition, according to Working Example 8 in Description 2 and evidence (Exhibit Ko 37), the introduction of the mutant-type yggB gene is found to cause the glutamic acid-producing ability of the strain to be enhanced as compared to a non-modified strain (it can be confirmed based on Working Example 8 and Exhibit Ko 37 that the glutamic acid-producing ability is enhanced in a configuration using the 19-type mutation that uses a mutant-type yggB gene having an amino acid sequence of SEQ ID NO: 22, as mentioned in 4. (3) B. below).

According to the points mentioned above and the entire import of oral arguments, it is found that, when Defendant's Production Process 1 is compared with Invention 2, the results of the comparison will be the same as the description in Attachment 7-1 "Comparison between Defendant's Production Processes 1 through 3 and Invention 2," and therefore the bacteria used in Defendant's Production Process 1 (Strain [ii] and Strain [x] which has the same characteristics as Strain [ii]) fall within the technical scopes of Inventions 2-1, 2-2, 2-3, and 2-4, and Defendant's Production Process 1, which uses those bacteria, falls within the technical scope of Invention 2-5.

(2) Regarding Defendant's Production Processes 2 and 3

The mutant-type yggB genes of the strains used in Defendant's Production Processes 2 and 3 (Strains [iii] through [ix] and [xi]) are those into which an A100T mutation has been introduced, as in the case with the yggB genes of the strains used in Defendant's Production Process 1 (Strains [ii] and [x]). Moreover, the Defendant has not alleged any differences between them with regard to the amino acid sequence of any other portions of the yggB genes. Therefore, also in regard to the mutant-type yggB genes of the strains used in Defendant's Production Processes 2 and 3 (Strains [iii] through [ix] and [xi]), it is found that the amino acid sequence before the mutation coincides with the amino acid sequence of SEQ ID NO: 6, and the amino acid sequence after the mutation coincides with the amino acid sequence of SEQ ID NO: 22, as in the case with the yggB gene of the strain used in Defendant's Production Process 1.

Accordingly, the results of comparison between the bacteria used in Defendant's Production Processes 2 and 3 and Invention 2 will be the same as those of the comparison between Defendant's Production Process 1 and Invention 2 mentioned in (1) above, and the bacteria used in Defendant's Production Processes 2 and 3 (Strains [iii] through [ix] and [xi]) all fall within the technical scopes of Inventions 2-1, 2-2, 2-3, and 2-4, while Defendant's Production Processes 2 and 3, which use those bacteria, both fall within the technical scope of Invention 2-5.

4. Regarding Issue 2-3 (whether Defendant's Production Process 4 falls within the technical scope of Invention 2 as an equivalent thereto)

(1) Regarding the criteria for determination of infringement under the doctrine of equivalents

Even if, within the configuration indicated in the claims, there is a part which is different from the product to be produced, etc. or the process to be used by the counterparty (hereinafter referred to as the "subject product, etc."), if [i] this part is not the essential part of the patented invention, [ii] the purpose of the patented invention can be achieved and an identical function and effect can be obtained by replacing this part with a part in the subject product, etc., [iii] a person with ordinary skill in the art of the invention (person skilled in the art) could have easily conceived of the abovementioned replacement at the time of the production, etc. of the subject product, etc., [iv] the subject product, etc. is not identical to publicly known art at the time of the patent application of the patented invention nor could have been easily conceived at that time by a person skilled in the art, and [v] there were no special circumstances such as the fact that the subject product, etc. had been intentionally excluded from the claims in the course of the patent application procedure of the patented invention, it is reasonable to construe that the subject product, etc. falls within the technical scope of the patented invention as being

an equivalent to the configuration indicated in the claims (see the judgment of the Third Petty Bench of the Supreme Court, 1994 (O) 1083, rendered on February 24, 1998, Minshu Vol. 52, No. 1, at 113 (hereinafter referred to as the "1998 Supreme Court Judgment"), and the judgment of the Second Petty Bench of the Supreme Court, 2016 (Ju) 1242, rendered on March 24, 2017, Minshu Vol. 71, No. 3, at 359 (hereinafter referred to as the "2017 Supreme Court Judgment"); hereinafter, the requirements in [i] through [v] above are respectively referred to as "first requirement" through "fifth requirement").

In addition, regarding the burden of alleging and proving the fulfillment of the first to fifth requirements, a person who alleges that the subject product, etc. is equivalent to a patented invention should be considered to have the burden of allegation and proof for the first to third requirements, while a person who denies the application of the doctrine of equivalents in relation to the subject product, etc. should be considered to have the burden of allegation and proof for the fourth and fifth requirements, which are related to the cases where the application of the doctrine of equivalents should be eliminated, even if the subject product, etc. is within the scope of equivalents under the first to third requirements (see the judgment of the Special Division of the Intellectual Property High Court, 2015 (Ne) 10014 rendered on March 25, 2016, Hanji No. 2306, at 87).

The following part examines whether Defendant's Production Process 4 falls within the technical scope of Invention 2 as an equivalent thereto, by first considering and comparing the contents of Invention 2 and the configuration using the 19-type mutation comprised therein and the contents of Defendant's Production Process 4, and then examining the first to fifth requirements of the doctrine of equivalents in order.

(2) Contents of Invention 2

A. Contents described in Description 2

The statements in the detailed explanation of the invention in Description 2 concerning Invention 2 are outlined as described in Attachment 13 "Statements in Description 2."

While paragraph [0033] of Description 2 has been corrected by Correction 2, as the correction of that part is found to be correction of errors as mentioned in 7. (1) B. below, the corrected statements are hereinafter used unless otherwise indicated.

B. Outline of Invention 2

According to the claims of Invention 2 (Attachment 5-1), the statements in Description 2 referred to in A. above, and the entire import of oral arguments, the outline of Invention 2 is found to be as follows.

(A) Technical field/background art

Invention 2 relates to the fermentation industry, and relates to a process for producing

L-glutamic acid, which is widely used as seasoning ingredients, etc., and further relates to a bacterium used in the process (paragraph [0001]).

L-glutamic acid has been conventionally produced on an industrial scale by the fermentation method using coryneform bacteria which have L-glutamic acid-producing ability, such as bacteria belonging to the genus *Brevibacterium* or the genus *Corynebacterium* (paragraph [0002]).

Generally, a wild-type strain of a coryneform bacterium does not produce glutamic acid under the condition of the presence of biotin. Accordingly, the production of L-glutamic acid by a coryneform bacterium is typically performed under a glutamic acid production-inducing condition such as a biotin-limited condition, a surfactant-added condition, and a penicillin-added condition. Further, as strains which can produce L-glutamic acid in the presence of sufficient biotin without applying these methods, a surfactant-temperature-sensitive strain, a penicillin-sensitive strain, a cerulenin-sensitive strain, and a lysozyme-sensitive strain, etc. have been developed. However, it was highly probable that these strains caused reduced adaptation to environmental changes in exchange for L-glutamic acid production. Therefore, considerable efforts have been required for the development of a strain capable of accumulating a significant amount of L-glutamic acid (paragraphs [0003] and [0004]).

On the other hand, a strain which produces L-glutamic acid in the presence of sufficient biotin can be achieved by deleting a gene encoding α-ketoglutarate dehydrogenase. However, the α-ketoglutarate dehydrogenase gene-deficient strain blocks the TCA cycle in the midway and thus grows slowly. Therefore, it was difficult to surely obtain a sufficient amount of bacterial cells, which was one of the problems (paragraph [0005]).

The yggB gene of a coryneform bacterium is a homologue of the yggB gene of *Escherichia coli* (*E. coli*), and has been analyzed as a kind of mechanosensitive channel, but the effect of the yggB gene on L-glutamic acid production was not known (paragraph [0006]).

(B) Problem to be solved by Invention 2

The problem to be solved by Invention 2 is to provide novel art to enhance the L-glutamic acid-producing ability of a coryneform bacterium when L-glutamic acid is produced by using the coryneform bacterium (paragraph [0007]).

(C) Means for solving the problem, etc.

a. Modification using the yggB gene

Invention 2 has clarified that the yggB gene is involved in the production of L-glutamic acid by a coryneform bacterium, and has found that the L-glutamic acid-

producing ability can be greatly enhanced by modifying the coryneform bacterium by using the yggB gene (paragraph [0008]).

The coryneform bacterium of Invention 2 is a coryneform bacterium having an L-glutamic acid-producing ability, wherein the coryneform bacterium has an enhanced L-glutamic acid-producing ability as compared to a non-modified strain as a result of being modified by using the yggB gene (paragraph [0011]).

Modification using the yggB gene includes a method of enhancing the expression level of the yggB gene and a method of introducing a mutant-type yggB gene, and Invention 2 uses the latter method (paragraph [0029]).

b. Introduction of a mutant-type yggB gene

Specific examples of the mutant-type yggB gene are shown below, but the mutant-type yggB gene is not particularly limited so far as it has a mutation that could enhance the L-glutamic acid producing ability of a coryneform bacterium under a condition in which an excess of biotin is present (paragraph [0069]).

(a) C-terminal side mutation

One mode of mutation that is introduced into the yggB gene in Invention 2 is a C-terminal side mutation, which is a mutation introduced into the C-terminal region of the yggB gene. This is a mutation which is introduced into a portion of the nucleotide sequence in the region encoding the sequence of amino acid numbers 419–533 of SEQ ID NOs: 6, 68, 84, and 85, or the sequence of amino acid numbers 419–529 of SEQ ID NO: 62 (paragraph [0070]). Examples of the C-terminal side mutation include [i] a mutation in which a transposable element is introduced (2A-1-type mutation) and [ii] a mutation in which the proline residue is substituted with another amino acid (66-type mutation, 22-type mutation) (Claim 1, paragraphs [0070] through [0072], Working Examples 2 through 6, Working Example 12, Working Example 13, and Working Example 17).

(b) Mutations in transmembrane regions

Another mode of mutation that is introduced into the yggB gene in Invention 2 is a mutation which is introduced into a portion encoding the five transmembrane regions which the YggB protein encoded by the yggB gene is presumed to have (in the amino acid sequence of the wild-type YggB protein of SEQ ID NOs: 6, 62, 68, 84, and 85, the transmembrane regions correspond to amino acid numbers 1–23 (the first transmembrane region), amino acid numbers 25–47 (the second transmembrane region), amino acid numbers 62–84 (the third transmembrane region), amino acid numbers 86–108 (the fourth transmembrane region), and amino acid numbers 110–132 (the fifth transmembrane region)). Examples of these mutations include [i] a mutation in the first transmembrane

region (A1-type mutation), [ii] a mutation in the fourth transmembrane region (19-type mutation), and [iii] a mutation in the fifth transmembrane region (L30-type mutation, 8-type mutation) (Claim 1, paragraphs [0073] through [0076], Working Examples 7 through 11, Working Example 15, and Working Example 16).

(D) Effect of Invention 2

L-glutamic acid can be efficiently produced by using a coryneform bacterium which has been modified by using the yggB gene of Invention 2 (paragraph [0010]).

C. Significance of Invention 2 (the principle for solving the problem)

In light of the above, it should be said that, when compared with the prior art described in Description 2, the specific technical idea in Invention 2 that cannot be found in the prior art (the principle for solving the problem) was to focus on the yggB gene of a coryneform bacterium, whose effect on production of glutamic acid had been unknown in the past, modify the YggB protein, which is a type of a mechanosensitive channel, by using a mutant-type yggB gene into which a C-terminal side mutation or mutations in transmembrane regions have been introduced, and thereby provide novel art to enhance the glutamic acid-producing ability of the bacterium.

D. Prior art as of Priority Date 2

Regarding prior art as of Priority Date 2, the Defendant alleges among other matters that, according to Exhibit Otsu 24 Document, it had already been known at that time that the osmoregulated channel (YggB) of *Corynebacterium glutamicum* contributed to efflux of glutamic acid.

(A) Contents described in Exhibit Otsu 24 Document

The contents described in Exhibit Otsu 24 Document include the following (Exhibit Otsu 24, the entire import of oral arguments; for Table 1, see Attachment 14 "Drawings of Cited Documents").

a. Abstract

(a) Lines 1 through 6 on page 572

"Bacteria respond to hypoosmotic stress by releasing low-molecular-mass solutes in order to maintain constant turgor pressure. We have studied the function of osmoregulated channel(s) in *Corynebacterium glutamicum*, which are responsible for efflux of various solutes upon sudden decrease in osmotic pressure. The channels preferentially mediated efflux of compatible solutes such as glycine betaine and proline. The release of molecules of similar size, e.g. glutamate or lysine, was restricted, and ATP was completely retained even after severe osmotic shock."

(b) Lines 13 through 14 on page 572

"These results indicate the presence of an osmoregulated channel in *C. glutamicum*

similar to the mechanosensitive channel(s) of *Escherichia coli*."

b. Results

(a) Table 1 (page 575)

"Table 1. Specificity of solute efflux after hypoosmotic shock in *C. glutamicum*. Cells were loaded with solutes under hyperosmotic conditions (1860 mOsm and 2100 mOsm, respectively) either by accumulation of labeled or unlabeled compatible solutes (glycine betaine, proline, ectoine), or by addition of dipeptides (lysine, alanine). The cells were diluted as given in the table. Efflux was measured 1 min after the hypoosmotic shock in both the supernatant and the pellet of the silicone oil centrifugation using scintillation counting (sc. count.), HPLC, enzymatic assay, NMR, luciferin/luciferase assay, or flame photometry (flame ph.). The values are in $\mu mol \cdot mgdm^{-1}$, since correcting to the true internal concentration according to the changed cytoplasmic volume would give misleading results of the net changes. All values are means of at least three experiments. For isoosmolar dilution and for hypoosmotic shock to 540 mOsm, relative values of retained solutes are given."

(b) Last line of the right column on page 575 through line 1 of the left column on page 576

"efflux of glutamate and lysine was significantly restricted"

c. Discussion

(a) Lines 38 through 49 of the left column on page 578

"As a soil bacterium, *C. glutamicum* is equipped with effective mechanisms responding to a frequent case of emergency, i.e. a hypoosmotic shock. The mechanical stress acting on the membrane due to osmotic changes became obvious by quantifying the significant change in cell size. The efflux system which we have studied, responds to hypoosmotic shock in a manner qualitatively similar to mechanosensitve channels which have previously been described in *E. coli* and in *L. plantarum* (Berrier et al., 1992; Schleyer et al., 1993; Glaasker et al., 1996). However, the *C. glutamicum* channel shows a number of specific properties, both with respect to transfer specificity and to regulation of efflux activity."

(b) Lines 30 through 33 of the right column on page 578

"Molecules related in size, e.g. glutamate, or even small inorganic ions (Na+, K+), obviously do not use this channel to the same extent as glycine betaine or proline."

(c) Lines 36 through 40 of the right column on page 578

"As long as the molecular identity of the channel in *C. glutamicum* is not known, however, the observed sequence of permeability through the *C. glutamicum* channel can also be explained by a multiplicity of channels with different specificities."

(d) Lines 46 through 55 of the right column on page 578

"The lack of sensitivity of this channel to Gd^{3+}, an unspecific channel blocker (Berrier et al., 1992; Schleyer et al., 1993; Hase et al., 1995), furthermore defines it as being different from the Gd^{3+}-sensitive MscL channel, described and cloned in *E. coli* (Sukharev et al., 1994). There are, however, other examples where this unspecific blocking agent did not work (Berrier et al., 1996). Thus, the channel in C. glutamicum may be similar to another type identified in *E. coli* by using electrophysiological techniques, i.e. the MscS channel (Martinac et al., 1987, 1990; Zoratti and Petronilli, 1988; Berrier et al., 1996)."

(e) Lines 39 through 46 of the left column on page 579

"Finally, it should be emphasized here that the described efflux channel is not related to the well-known glutamate efflux observed in *C. glutamicum* under particular metabolic conditions. Although glutamate efflux under conditions of continuous production of this amino acid seems to respond to osmotic changes with respect to its activity (Lambert et al., 1995), it is mediated by an energy-dependent, specific carrier system, as has been shown before (Gutmann et al., 1992; Kramer, 1994)."

(B) Examination

As mentioned above, Table 1 of Exhibit Otsu 24 Document describes that, as a result of measuring efflux of solutes when hypoosmotic shock was applied to *Corynebacterium glutamicum*, efflux of glutamic acid was observed under hypoosmotic conditions. However, the efflux was only 20% at 540 mOsm, which greatly differed from the efflux rates of substances such as glycine betaine (70% efflux under the same conditions) and proline (71% efflux under the same conditions). The abovementioned efflux rate of glutamic acid was the lowest following ATP, for which efflux was hardly observed, among the total of 11 types of solutes examined, and it was assessed that "efflux of glutamate and lysine was significantly restricted."

In light of such results, Exhibit Otsu 24 Document states that osmoregulated channels in *Corynebacterium glutamicum* preferentially mediated efflux of glycine betaine and proline, but that the channels were unrelated to efflux of glutamic acid, and derived a conclusion that efflux of glutamic acid is mediated not by osmoregulated channels, but by a carrier system as had been indicated before.

Accordingly, although Exhibit Otsu 24 Document suggests that *Corynebacterium glutamicum* has osmoregulated channels having similar characteristics as mechanosensitive channels of *E. coli*, it does not show that the osmoregulated channels contribute to efflux of glutamic acid.

In addition, although other documents cited by the Defendant as common general

technical knowledge as of Priority Date 2 (Exhibits Otsu 28 and 37 through 40) also have some parts that mention the need to increase the amount of amino acid efflux to outside of the bacterial cell when producing amino acid by using a coryneform bacterium, they are not found to mention the relevance of the osmoregulated channel (YggB) of a coryneform bacterium to glutamic acid efflux.

Consequently, the statements on prior art in Description 2, including the fact that the yggB gene of a coryneform bacterium is a homologue of the yggB gene of *E. coli*, and has been analyzed as a kind of mechanosensitive channel, but the effect of the yggB gene on L-glutamic acid production was not known, (paragraphs [0002] through [0006]) are not found to be insufficient from an objective viewpoint.

(3) Regarding the configuration using the 19-type mutation

A. Contents of the configuration using the 19-type mutation

The configuration using the 19-type mutation is a configuration comprised in Invention 2-5, and it is the process for producing glutamic acid claimed in Claim 11 of Patent 2, which uses a coryneform bacterium into which the mutant-type yggB gene mentioned in (e) of Claim 6, which cites Claim 1 or 4, has been introduced.

Among the mutations introduced in the yggB gene in Invention 2, the 19-type mutation introduces a mutation in the portion encoding the fourth transmembrane region of the YggB protein (paragraph [0075]). Specifically, it is a mutation in which alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 encoded by the yggB gene derived from *Corynebacterium glutamicum* is substituted with threonine (A100T mutation), and the amino acid sequence encoded by the yggB gene after the mutation is SEQ ID NO: 22 (paragraphs [0033], [0075], and [0119]).

B. Effect of the configuration using the 19-type mutation

As the Defendant alleges that, among the configurations comprised in Invention 2, the configuration using the 19-type mutation only marginally affects the glutamic acid production amount if at all, the effect of the configuration using the 19-type mutation is examined below.

(A) Regarding Working Example 10

In Working Example 10, in all cases under a glutamic acid production-inducing condition, such as a surfactant (Tween40)-added condition or a biotin-limited condition, the glutamic acid production amount was found to increase as compared to a wild-type strain (non-modified strain) due to the configuration using the 19-type mutation ([Table 9] and [Table 10]).

(B) Regarding Working Example 8

a. Regarding statements in Description 2

Working Example 8 is an experiment performed, as in the case with Working Example 2, under a non-inducing condition where no surfactant, etc. is added, in the presence of 300 μg/l biotin, which corresponds to "a condition with an excess of biotin" (paragraph [0032]) (paragraphs [0120] and [0097]).

[Table 7] of Working Example 8 shows that the glutamic acid production amount of a wild-type strain (non-modified strain) was 0.5 g/L, whereas that of a strain into which the 19-type mutation was introduced was 0.7 g/L. Regarding this result, paragraph [0120] contains the description that the glutamic acid accumulation in the culture solution was greatly enhanced for the strain into which the 19-type mutation was introduced as compared to the wild-type strain.

b. The Defendant alleges that the glutamic acid production amount of the abovementioned strain into which the 19-type mutation was introduced is so low that it would match the blank values in Working Example 2 and Working Example 3, and the difference with the wild type is also only within the margin of error.

According to paragraph [0097] and Exhibit Ko 37, it can be found that the blank value indicates the value of the glutamic acid which is derived from the soybean hydrolysate added to the medium as a nutrient for culturing the bacterial cell and which is contained in the medium at the start of culture, but in [Table 1], [Table 2], [Table 4], and [Table 5] of Working Examples 2, 3, and 6, all of which are said to have adopted the culture method of Working Example 2, different values from 0.4 g/L to 0.7 g/L are indicated as blank values. Accordingly, even if the same culture method of Working Example 2 is adopted, the blank value could differ depending on the experiment, and hence it cannot be said that there was no enhancement in the glutamic acid production amount of the 19-type mutation-introduced strain in Working Example 8 by comparing the amount with blank values in different experiments. In addition, it also cannot be said that the difference in the glutamic acid production amount between the 19-type mutation-introduced strain and the wild-type strain in Working Example 8 was within the margin of error, based on blank values in different experiments or based on the culture results of the same strain in different experiments.

c. Regarding the experiment in Exhibit Otsu 44

The Defendant alleges that, in the experiment of Exhibit Otsu 44 that was performed on request by the Defendant or CJCJ, no enhancement in the glutamic acid-producing ability caused by the 19-type mutation was observed.

Exhibit Otsu 44 contains a description that, as a result of performing a culture experiment under the same medium conditions as Working Example 8, using the same strains as in Working Example 8, namely, a wild-type ATCC13869 strain and a

ATCC13869 strain into which the 19-type mutation-introduced yggB gene was introduced, it was confirmed that neither strain produces glutamic acid.

While Working Example 8 adopts the same culture method as Working Example 2 (paragraph [0120]), Working Example 2 contains a description of "shaking culture" in a "flask medium," but it does not specify the type of flask or the shaking speed (paragraph [0097]), and there is no description in Working Example 8 that specifies them either.

According to the evidence (Exhibits Ko 37 through 43) and the entire import of oral arguments, it is found, as the common general technical knowledge as of Priority Date 2, that the glutamic acid production by fermentation will be inhibited unless the oxygen supply amount is sufficient, and that, while a conical flask, baffled conical flask, and Sakaguchi flask are typically used for shaking culture of aerobic bacteria, in order to supply sufficient oxygen, higher speed shaking will be required for a conical flask compared to a Sakaguchi flask.

While in the experiment of Exhibit Otsu 44, the shaking culture is found to have been performed at a speed of 115 rpm using a conical flask (Exhibit Otsu 44; the entire import of oral arguments), Description 2 has a statement in paragraph [0146] of Working Example 15 about an experiment in which shaking culture was performed at a speed of 115 rpm, but as it is stated that a Sakaguchi flask was used in the experiment, it cannot be said that the abovementioned shaking speed in Exhibit Otsu 44 was appropriate based on the statement in paragraph [0146].

In the experiment in 1. of Exhibit Ko 37 conducted by the Plaintiff, shaking culture was performed at a speed of 115 rpm using the same medium as in Working Example 8 and using a Sakaguchi flask. As a result, it was confirmed that the glutamic acid-producing ability of a 19-type mutation-introduced ATCC13869 strain was enhanced compared to a wild-type ATCC13869 strain. Moreover, when the same experiment was conducted by changing the conditions to those of Exhibit Otsu 44, where a conical flask is used and a 115 rpm shaking speed is adopted, it was confirmed that the glutamic acid production amount of the 19-type mutation-introduced ATCC13869 strain substantially declined as compared to the abovementioned experiment results using a Sakaguchi flask. Taking these factors into consideration, it can be considered that the glutamic acid production was inhibited in the experiment of Exhibit Otsu 44 due to the low shaking speed, so the results of that experiment cannot be regarded to affect the contents of Working Example 8 described in a. above.

d. Consequently, as described in Working Example 8, the glutamic acid production is found to increase as compared to a wild-type strain (non-modified strain) due to the configuration using the 19-type mutation under a condition with an excess of biotin.

(C) According to (A) and (B) above, the configuration using the 19-type mutation is found to have an effect of solving the problem to be solved by Invention 2, and the Defendant's argument that its effect is marginal cannot be adopted.

(4) Regarding the strains to be used in Defendant's Production Process 4

A. Regarding introduction of the A98T mutation and V241I mutation into a yggB gene derived from *Corynebacterium callunae*

According to the evidence (Exhibit Ko 54, Exhibit Otsu 4) and the entire import of oral arguments, it is found that a mutant-type yggB gene derived from wild-type *Corynebacterium callunae* is introduced into Strain [xiii], and that the mutant-type yggB gene is one into which the following mutations have been introduced: a mutation in which alanine at position 98 in the amino acid sequence encoded by the yggB gene of wild-type *Corynebacterium callunae* (the amino acid sequence of YggB of the DSM20147 strain, which is wild-type *Corynebacterium callunae*) is substituted with threonine (A98T mutation) and a mutation in which valine at position 241 in that amino acid sequence is substituted with isoleucine (V241I mutation).

The Defendant alleges that, although it has not submitted analysis results concerning the yggB gene derived from *Corynebacterium callunae*, which was introduced into Strain [xii], the yggB gene also had the A98T mutation and V241I mutation. As the Defendant has not asserted that there were any other points that differed from the abovementioned mutant-type yggB gene which was introduced into Strain [xiii], it is reasonable to find that the same mutant-type yggB gene was being introduced into Strain [xii] as well.

B. Introduction of mutations into a yggB gene derived from *Corynebacterium glutamicum*

According to the evidence (Exhibit Ko 54, Exhibits Otsu 2 and 4) and the entire import of oral arguments, it is also found that a sequence of a yggB gene derived from *Corynebacterium glutamicum*, which differed from the mutant-type yggB gene in A. above, also existed in Strains [xii] and [xiii], and that the amino acid sequence encoded by the yggB gene derived from *Corynebacterium glutamicum* had characteristics of ● (omitted)●.

C. Effect of introduction of each mutation on the glutamic acid-producing ability

While Strains [xii] and [xiii] are both *Corynebacterium glutamicum* (the entire import of oral arguments), how their glutamic acid-producing ability is affected by the introduction of the mutations mentioned in A. and B. above into the yggB genes of these strains is examined below.

(A) Regarding the A98T mutation and V241I mutation

a. Results of the experiment of Exhibit Ko 92 conducted by the Plaintiff

In the experiment conducted by the Plaintiff, when [i] a mutant-type yggB gene

obtained by introducing only the A98T mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) was introduced on the chromosome of *Corynebacterium glutamicum*, and shaking culture was performed at a speed of 200 rpm (the same speed as in the Defendant's experiment mentioned in c. below) using the same medium as in Working Example 8 (a condition with an excess of biotin) and using a baffled conical flask, it was confirmed that the glutamic acid production amount of that strain increased compared to *Corynebacterium glutamicum* into which a wild-type yggB gene derived from *Corynebacterium callunae* has been introduced (Experiment 1 of Exhibit Ko 92).

In addition, as a result of performing shaking culture using the abovementioned Strain [i] and using the same medium as in Working Example 10 (a condition in which surfactant Tween 40 has been added), it was confirmed that the glutamic acid production amount of the abovementioned Strain [i] increased compared to *Corynebacterium glutamicum* into which a wild-type yggB gene derived from *Corynebacterium callunae* has been introduced (Experiment 2 of Exhibit Ko 92).

b. Results of the experiment of Exhibit Ko 54 conducted by the Plaintiff

In the experiment conducted by the Plaintiff, when [ii] a mutant-type yggB gene obtained by introducing the A98T mutation and V241I mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) and [iii] a mutant-type yggB gene obtained by introducing only the V241I mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) were each introduced into *Corynebacterium glutamicum* as plasmids, and shaking culture was performed at a speed of 115 rpm using the same medium as in Working Example 8 (a condition with an excess of biotin) and using a Sakaguchi flask, it was confirmed that, compared with *Corynebacterium glutamicum* into which a wild-type yggB gene derived from *Corynebacterium callunae* has been introduced as a plasmid, the glutamic acid production amount of the abovementioned Strain [iii] remained the same with both strains hardly producing any glutamic acid, but the glutamic acid production amount of the abovementioned Strain [ii] greatly increased (Experiment 2 of Exhibit Ko 54).

c. Results of the experiment of Exhibit Otsu 97

In an experiment conducted by the Defendant's affiliated research institution, when a mutant-type yggB gene obtained by introducing only the A98T mutation into a yggB gene derived from *Corynebacterium callunae* was introduced into *Corynebacterium glutamicum* as a plasmid, and shaking culture was performed at a speed of 200 rpm using a medium containing an excess of biotin (300 μg/l) and using a baffled conical flask, no glutamic acid production was confirmed in the strain (Exhibit Otsu 97).

d. Examination

(a) Comparison between the Plaintiff's experiment and the Defendant side's experiment

As mentioned in a. and c. above, different results are indicated in the Plaintiff's experiment (Exhibit Ko 92) and the Defendant side's experiment (Exhibit Otsu 97) concerning whether a mutant-type yggB gene obtained by introducing only the A98T mutation into a yggB gene derived from *Corynebacterium callunae* has an effect of enhancing the glutamic acid-producing ability of the strain under a condition in which an excess of biotin is present.

With regard to this difference, the report of the Plaintiff's experiment mentioned above states in its discussion part that, in the Defendant side's experiment, a mutant-type yggB gene was introduced as a plasmid, but no confirmation was made as to whether the introduced plasmid was maintained in a stable manner within the bacterial cell, so it is probable that the effect of the mutant-type yggB gene could not be confirmed as a result of the plasmid becoming unstable (Exhibit Ko 92).

While the method to introduce a mutant-type yggB gene in Invention 2 includes a method to introduce the yggB gene on the chromosome of a bacterium and a method to introduce a plasmid containing a mutant-type yggB gene into the bacterial cell (paragraph [0050]), it has been pointed out that generally when introducing a genetic mutation by using a plasmid, there are problems such as loss of the plasmid itself (paragraphs [0002] and [0003] of Description 1). Also taking into consideration that the Defendant has not offered specific allegations or proof concerning the stability of the mutant-type yggB gene introduced as a plasmid in the Defendant side's experiment above, the Defendant side's experiment results (Exhibit Otsu 97) cannot be adopted with regard to the effect of the case where only the A98T mutation is introduced into a yggB gene derived from *Corynebacterium callunae*.

(b) Effect of introduction of the A98T mutation and V241I mutation

Thus, according to the experiment results in a. and b. above, the introduction of a mutant-type yggB gene obtained by introducing the A98T mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) is found to cause the glutamic acid-producing ability of *Corynebacterium glutamicum* to be enhanced under a condition with an excess of biotin, whereas the introduction of the V241I mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) is found not to contribute to enhancement of the glutamic acid-producing ability of *Corynebacterium glutamicum* under a condition with an excess of biotin.

The Defendant asserts that the introduction of only the A98T mutation into a yggB gene derived from *Corynebacterium callunae* does not cause the glutamic acid-producing

ability to be enhanced, but that the glutamic acid-producing ability increases only when the V241I mutation is added to the A98T mutation. However, in light of the experiment results in a. and b. above, this assertion cannot be adopted.

(B) Regarding introduction of a mutation into a yggB gene derived from *Corynebacterium glutamicum*

According to the evidence (Exhibit Ko 54), it is found that when, after introducing the mutation mention in B. above into a yggB gene derived from *Corynebacterium*, this mutant-type yggB gene was introduced into *Corynebacterium glutamicum*, and shaking culture was performed at a speed of 115 rpm using the same medium as in Working Example 8 (a condition with an excess of biotin) and using a Sakaguchi flask, the glutamic acid-producing ability could not be confirmed in this strain, indicating the same result as for a strain without the yggB gene; and with regard to this experiment result, a consideration has been made that the amino acid sequence encoded by the abovementioned mutant-type yggB gene does not function as YggB. Consequently, it can be said that the introduction of this mutation does not contribute to enhancement of the glutamic acid-producing ability under a condition in which an excess of biotin is present.

(5) Comparison between Defendant's Production Process 4 and Invention 2

A. In light of the examination made in (4) above, it can be said that Strains [xii] and [xiii] used in Defendant's Production Process 4 are both *Corynebacterium glutamicum* into which a mutant-type yggB gene obtained by introducing the A98T mutation and V241I mutation into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) was introduced, and their glutamic acid-producing ability under a condition in which an excess of biotin is present has been enhanced as compared to a non-modified strain.

Consequently, Strains [xii] and [xiii] used in Defendant's Production Process 4 literally fulfill Constituent Features 2-A and 2-B of Invention 2-1 but do not fulfill Constituent Feature 2-C of Invention 2-1, and they do not fulfill any of the constituent features (Constituent Features 2-D, 2-E, 2-F-1, 2-F-2, and 2-F-3) of Inventions 2-2 and 2-3. In addition, according to the entire import of oral arguments, as in the case with Defendant's Production Processes 1 through 3, Defendant's Production Process 4 is found to literally fulfill Constituent Feature 2-H of Invention 2-5, except for the parts citing Claims 1 through 10, and fulfill Constituent Features 2-I and 2-J of Invention 2-5.

B. Differences between Defendant's Production Process 4 and the configuration using the 19-type mutation

In light of the examination made in (3) and (4) above, the differences between Defendant's Production Process 4 and the configuration using the 19-type mutation comprised in Defendant's Invention 2-5 (the process for producing glutamic acid claimed

in Claim 11 of Patent 2, which uses a coryneform bacterium into which the mutant-type yggB gene mentioned to in (e) of Claim 6, which cites Claim 1 or 4, has been introduced) lie in the parts relating to Constituent Features 2-C, 2-D, 2-E, 2-F-1, 2-F-2, 2-F-3, and 2-H (literally non-fulfilling parts mentioned in A. above), and specifically, the following differences are found with regard to the strains being used.

Difference 1: The mutant-type yggB gene introduced is one derived from *Corynebacterium glutamicum* (the amino acid sequence before the mutation is SEQ ID NO: 6) in the case of the strain with the configuration using the 19-type mutation, whereas it is one derived from *Corynebacterium callunae* (DSM20147 strain) in the case of the strains used in Defendant's Production Process 4 (Strains [xii] and [xiii]).

Difference 2: The mutation introduced into the yggB gene is one in which alanine at position 100 in the amino acid sequence encoded by the yggB gene is substituted with threonine (A100T mutation) in the case of the strain with the configuration using the 19-type mutation, whereas it is one in which alanine at position 98 in the amino acid sequence encoded by the yggB gene is substituted with threonine (A98T mutation) in the case of the strains used in Defendant's Production Process 4.

Difference 3: In the case of the configuration using the 19-type mutation, only the A100T mutation is introduced into the yggB gene, whereas in the case of the strains used in Defendant's Production Process 4, not only the A100T mutation, but also a mutation in which valine at position 241 in the amino acid sequence encoded by the yggB gene is substituted with isoleucine (V241I mutation) is introduced into the yggB gene.

As mentioned in (4) B. above, apart from the abovementioned mutant-type yggB gene derived from *Corynebacterium callunae*, Strains [xii] and [xiii] have a sequence of a yggB gene derived from *Corynebacterium glutamicum*, and the amino acid sequence encoded by it is ●(omitted)●. However, as mentioned in (4) C. (B) above, it is considered that the sequence of this yggB gene does not contribute to enhancement of the glutamic acid-producing ability under a condition in which an excess of biotin is present, and that the amino acid sequence encoded by it does not function as YggB, and neither party argues that the presence of that sequence is a difference that should be considered in determining whether or not infringement under the doctrine of equivalents is established. Therefore, it cannot be said that the presence of this sequence is a difference that should be considered in addition to Differences 1 through 3 above.

(6) Regarding the first requirement (non-essential part)

A. Essential part of Invention 2 (configuration using the 19-type mutation)

As mentioned above, in this case, the reasonable approach to determine whether or not Defendant's Production Process 4 falls within the technical scope of Invention 2 as an

equivalent thereof would be to compare Defendant's Production Process 4 with the configuration using the 19-type mutation comprised in Invention 2, thus, also in examining the essential part of Invention 2 in relation to fulfillment of the first requirement, specifically, the essential part of the configuration using the 19-type mutation will be examined as follows.

(A) The substantial value of an invention which the Patent Act intends to protect exists in the disclosure, with a specific structure, to society of a means for solving a technical problem that could not have been solved by prior art, which is based on a unique technical idea that is not seen in prior art. The essential part of a patented invention should be understood as the characteristic part which constitutes a unique technical idea that is not seen in prior art in the statements in the claims of the patented invention (see the abovementioned Intellectual Property High Court judgment of March 25, 2016).

(B) As mentioned in (2) C. above, when compared with the prior art described in Description 2, the unique technical idea in Invention 2 that is not seen in prior art (the principle for solving the problem) should be considered to be the following: to focus on the yggB gene of a coryneform bacterium, whose effect on production of glutamic acid had been unknown in the past, modify the YggB protein, which is a type of a mechanosensitive channel, by using a mutant-type yggB gene into which a C-terminal side mutation or mutations in transmembrane regions are introduced, and thereby provide novel art to enhance the glutamic acid-producing ability of the bacterium. In addition, as examined in (2) D. above, the statements on prior art in Description 2 are not found to be insufficient from an objective viewpoint.

(C) As mentioned in (3) A. above, the configuration using the 19-type mutation is comprised in Invention 2-5, and it uses a coryneform bacterium into which the mutant-type yggB gene mentioned in (e) of Claim 6, which cites Claim 1 or 4, has been introduced. In light of the unique technical idea or the principle for solving the problem in Invention 2 mentioned in (B) above, the essential part of the configuration using the 19-type mutation is found to be "to introduce into a yggB gene derived from a coryneform bacterium a mutation equivalent to the A100T mutation in the yggB gene derived from *Corynebacterium glutamicum*, modify the coryneform bacterium by using that mutant-type yggB gene, and enhance the glutamic acid-producing ability of the bacterium even under a condition in which an excess of biotin is present.

(D) The Defendant alleges that, in light of the application history, the state of the art as of Priority Date 2, and the effect of the configuration using the 19-type mutation, when determining the essential part of the configuration using the 19-type mutation, the statements of the claims should not be adopted as a generic concept, but instead should

be limited to "the A100T mutation in the specific amino acid sequence of SEQ ID NO: 22, which is the sequence of the yggB gene after the mutation" described in the claims.

However, according to the contents of Description 2 described in (2) A. and B. above, Invention 2 does not target a problem that only exists in a coryneform bacterium having a yggB gene with a specific sequence, and the principle for solving the problem was to introduce novel art of modifying YggB protein, which is a type of a mechanosensitive channel, by using a mutant-type yggB gene into which a C-terminal side mutation or mutations in transmembrane regions are introduced, in order to enhance the glutamic acid-producing ability. Even by taking into consideration that Claim 1 or Claim 4 of Invention 2 lists the amino acid sequences of the yggB gene before introduction of the mutation, and Claim 6 of Invention 2 lists the amino acid sequences of the yggB gene after the mutation, when determining Invention 2 and the essential part of the configuration using the 19-type mutation comprised therein, it is reasonable to find that the species of the coryneform bacterium from which the yggB gene is derived, the specific sequence of the entire yggB gene before the mutation, or the specific sequence of the yggB gene after the introduction of a mutation equivalent to the A100T mutation is not an essential part. The same applies even if consideration is given to the following facts, which are pointed out by the Defendant: the fact that initial Claim 1 at the time of filing the application for Patent 2 did not specify the species of the coryneform bacterium from which the yggB gene is derived or the amino acid sequence of the yggB gene before and after the mutation, but rather, as a result of correction, the amino acid sequences of the yggB gene were specified as those of SEQ ID NOs: 6, 62, 68, 84, and 85, which are derived from *Corynebacterium glutamicum* (including *Brevibacterium flavum*) or *Corynebacterium melassecola*, as in current Claim 1 (paragraph [0033]; Exhibits Otsu 80 through 84); and the fact that the amino acid sequences of SEQ ID NOs: 6, 62, 68, 84, and 85, which are described in Claim 1, are mutually highly homologous (Exhibit Otsu 85). In addition, the Defendant alleges that the statements of the claims after the re-correction of Patent 2 should also be taken into consideration in relation to the application history, but it should be said that the contents of that correction at least do not affect the determination of the essential part of Invention 2 before the correction.

Moreover, the Defendant's allegations regarding the state of the art as of Priority Date 2 and the effect of the configuration using the 19-type mutation cannot be adopted as mentioned in (2) D. and (3) B. above, and it cannot be said that the essential part of the configuration using the 19-type mutation should be limited to the specific sequence of the yggB gene before and after the mutation described in the claims based on those allegations. Thus, the Defendant's allegations on those points do not affect the

determination mentioned in (C) above either.

B. Regarding Difference 1

As mentioned in A. (D) above, with regard to the essential part of the configuration using the 19-type mutation, when considering that it is reasonable to find that the species of the coryneform bacterium from which the yggB gene is derived, the specific sequence of the entire yggB gene before the mutation, or the specific sequence of the yggB gene after the introduction of a mutation equivalent to the A100T mutation is not an essential part of the configuration, and when also considering the points in (A) and (B) below, it cannot be said that the differences relating to Difference 1, that is, the difference in the species of bacterium from which the introduced mutant-type yggB gene is derived and the resulting difference in the specific sequence of the yggB gene constitute the essential part of the configuration using the 19-type mutation.

(A) Characteristics of *Corynebacterium callunae*

According to the evidence (Exhibits Ko 54 through 56 and 78, Exhibit Otsu 86) and the entire import of oral arguments, it is found that, *Corynebacterium callunae*, when compared with *Corynebacterium glutamicum*, belongs to the same genus *Corynebacterium* and is also a closely related bacterium in that category, and as in the case with *Corynebacterium glutamicum*, it is known as a microorganism used when industrially producing glutamic acid. In Description 2 as well, it is mentioned as an example of the coryneform bacterium in Invention 2 (paragraph [0012]). Exhibit Otsu 96 describes that, although the two bacteria belong to the same genus *Corynebacterium*, their characteristics are varied, but it does not indicate that there are specific differences between the characteristics of *Corynebacterium callunae* and those of *Corynebacterium glutamicum* in relation to glutamic acid production, and thus does not affect the abovementioned determination.

(B) Identity and homology levels of the specific sequence of the mutant-type yggB gene

According to the evidence (Exhibits Ko 54, 56, and 71, Exhibit Otsu 85) and the entire import of oral arguments, when the entire amino acid sequence of the mutant-type yggB gene derived from *Corynebacterium glutamicum* used in the configuration using the 19-type mutation (the sequence of SEQ ID NO: 22 after the introduction of the A100T mutation) is compared with the entire amino acid sequence of the yggB gene of Strains [xii] and [xiii], it is found that these amino acid sequences are approximately 64% identical, and when they are compared by also taking into consideration conservative substitution with a similar amino acid, the sequences are approximately 91% homologous.

C. Regarding Difference 2

According to the evidence (Exhibit Ko 54, Exhibit Otsu 85) and the entire import of

oral arguments, it is found that, when comparison is made by a general method for comparing amino acid sequences for the amino acid sequences of the yggB gene derived from *Corynebacterium glutamicum* (the amino acid sequence of SEQ ID NO: 6 before the mutation) and the yggB gene derived from *Corynebacterium callunae* (DSM20147 strain), which were introduced into a strain with the configuration using the 19-type mutation, alanine at position 100 in the former corresponds to alanine at position 98 in the latter. In addition, when these alanine are compared using three-dimensional protein structures, they are found to exist at equivalent positions in the transmembrane regions of the YggB.

Accordingly, with regard to Difference 2, the fact that the position of alanine to be substituted with threonine in the amino acid sequence encoded by the yggB gene is alanine at position 100 in the case of a strain with the configuration using the 19-type mutation, whereas it is alanine at position 98 in Strains [xii] and [xiii] of Defendant's Production Process 4, does not constitute a different in the essential part of the configuration using the 19-type mutation.

D. Regarding Difference 3

(A) According to the evidence (Exhibits Ko 54 and 56, Exhibit Otsu 85) and the entire import of oral arguments, when comparison is made by a general method for comparing amino acid sequences as in C. above, the following results are found: that valine at position 241 in the yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) corresponds to valine at position 243 in the yggB gene of a strain with the configuration using the 19-type mutation (amino acid sequence of SEQ ID NO: 6 before the mutation); that these valines do not correspond to the amino acids in the transmembrane region (paragraph [0073]) and the C-terminal side region (paragraph [0070]) disclosed in Description 2, and even when the three-dimensional protein structures are examined, they do not exist in the transmembrane regions or the vicinities thereof; and that as valine and isoleucine have close characteristics, the substitution of valine with isoleucine has little effect on the protein structure.

(B) In addition, Description 2 contains a description that, as long as there is a function to enhance the L-glutamic acid-producing ability under a condition in which an excess of biotin is present, there may be "an amino acid sequence in which one or several amino acids in addition to amino acids of the ... mutation point" of the C-terminal side mutation or the transmembrane region "are substituted, deleted, inserted, or added," that this substitution is "preferably a conservative substitution (neutral mutation)," and that its examples include substitution of valine (val) with isoleucine (ile) (paragraph [0078]). Furthermore, Claim 6 (f) indicates that "DNA that encodes a protein having an amino acid

sequence in which one to five amino acids" in the amino acid sequence of the mutant-type yggB gene (SEQ ID NO: 22) of the strain with the configuration using the 19-type mutation "are substituted, deleted, inserted, or added, and, through introduction into a coryneform bacterium, enhances the L-glutamic acid-producing ability of the coryneform bacterium when cultured in a medium containing an excess of biotin" is also comprised in Invention 2-3.

(C) Moreover, as mentioned in (4) C. above, it is found that, while Strains [xii] and [xiii] into which the A98T mutation and V241I mutation are introduced show enhancement in the glutamic acid-producing ability under a condition with an excess of biotin, which solves the problem to be solved by Invention 2, what contributes to this is the A98T mutation, and not the V241I mutation.

(D) In light of these points, the difference relating to Difference 3, that is, the fact that, in addition to the A98T mutation relating to Difference 2, the V241I mutation is introduced into the strain of Defendant's Production Process 4, is a difference to make a conservative substitution to one amino acid at a site unrelated to the mutations in the transmembrane regions or the C-terminal side mutation, which constitutes the principle for solving the problem to be solved by Invention 2 disclosed in Description 2, and addition of this mutation to the A98T mutation does not affect the solution of the problem. Therefore, this difference is not a difference in the essential part of the configuration using the 19-type mutation.

E. Consequently, as none of the Differences 1 through 3 between the configuration using the 19-type mutation and Defendant's Production Process 4 are differences in the essential part of the patented invention, Defendant's Production Process 4 that uses Strains [xii] and [xiii] is found to fulfill the first requirement of the doctrine of equivalents.

(7) Regarding the second requirement (replaceability)

According to (3) B. and (4) C. above, it is found that, Strains [xii] and [xiii] used in Defendant's Production Process 4 show enhancement in the glutamic acid-producing ability, which solves the problem to be solved by Invention 2, and have the same function and effect as the configuration using the 19-type mutation, which is to enhance the glutamic acid-producing ability under a condition with an excess of biotin.

The Defendant alleges that, as the essential part of the configuration using the 19-type mutation is a specific mutation, SEQ ID NO: 22, and its function and effect are also limited to the function and effect of a specific amino acid sequence, SEQ ID NO: 22, the function and effect of Defendant's Production Process 4 and those of the configuration using the 19-type mutation are not the same.

However, as mentioned in (6) A. (D) above, in light of the problem to be solved by

Invention 2 and the principle for solving that problem, it cannot be said that the essential part of the configuration using the 19-type mutation should be limited to a specific sequence of the yggB gene before and after the mutation, and it cannot be said either that the function and effect of the configuration using the 19-type mutation are limited to those of the amino acid sequence of SEQ ID NO: 22. Therefore, the Defendant's allegation cannot be adopted.

Consequently, Defendant's Production Process 4 that uses Strains [xii] and [xiii] is found to fulfill the second requirement of the doctrine of equivalents.

(8) Regarding the third requirement (ease of replacement)

A. Regarding Difference 1 and Difference 2

(A) Statement of *Corynebacterium callunae* in Description 2

In Description 2, *Corynebacterium callunae* is mentioned as an example of the coryneform bacterium in Invention 2, as in the case with *Corynebacterium glutamicum* (including *Brevibacterium flavum*) or *Corynebacterium melassecola* from which SEQ ID NOs: 6, 62, 68, 84, and 85 of the yggB gene described in Claim 1 are derived (paragraphs [0012] and [0033]).

(B) Common general technical knowledge concerning the characteristics of *Corynebacterium callunae*

According to the evidence (Exhibits Ko 54 through 56 and 78, Exhibit Otsu 86) and the entire import of oral arguments, *Corynebacterium callunae* is found to have been known from long ago by persons skilled in the art as a microorganism used when industrially producing glutamic acid, with a U.S. patent being registered for a process for producing glutamic acid by using *Corynebacterium callunae* in 1963, for example. In addition, with regard to the relationship between *Corynebacterium callunae* and *Corynebacterium glutamicum*, it was already known as of Priority Date 2 that they both belong to the same genus *Corynebacterium* and are also closely related bacteria in that category, and even after the classification was revised due to discovery of new coryneform bacteria or the like, this point is found to have remained the same. It is found that these characteristics of *Corynebacterium callunae* were already common general technical knowledge as of July 2016, when production by Defendant's Production Process 4 was started.

(C) Status of genomic analysis of *Corynebacterium callunae*

According to the evidence (Exhibits Ko 56, 74, and 84, Exhibit Otsu 86) and the entire import of oral arguments, it is found that, the entire genome of the *Corynebacterium callunae* DSM20147 strain and the amino acid sequence of the yggB gene had not been analyzed as of Priority Date 2, but these analyses were completed by March 2013, and

the results of both analyses were registered on the database in the same month. It is also found that the results of the abovementioned genomic analysis of the DSM20147 strain were reported in an article published in January 2015 (*Standards in Genomic Sciences* (2015) 10：5; Exhibit Otsu 86), which described the relationship with *Corynebacterium glutamicum* as shown below, and suggested the possibility that knowledge concerning *Corynebacterium glutamicum* may be applied to *Corynebacterium callunae* due to the closeness of their genome sequences.

a. Last line of the left column to line 6 of the right column on page 5

"As *C. callunae* was shown to produce l-glutamate in an amount comparable to *C. glutamicum*, *C. callunae* might be considered as a potential candidate for future genome reduction efforts since the chromosome is already considerably smaller than that of *C. glutamicum* and *C. efficiens* (2.84 Mbp versus 3.21 Mbp and 3.15 Mbp, respectively)."

b. Line 10 to line 14 of the left column on page 6

"Therefore, this bacterium might be an ideal choice for future development of a platform strain as the otherwise high degree of similarity of its genome content to the well studied *C. glutamicum* would allow an easy transfer of knowledge to the new host."

(D) Results of analysis of the yggB gene of *Corynebacterium callunae* using general software, etc. after the genomic analysis

According to the evidence (Exhibit Ko 54, Exhibits Otsu 85 and 98) and the entire import of oral arguments, as mentioned in (C) above, as of 2013, when the yggB gene of *Corynebacterium callunae* was registered on the database, it was possible to search, from among the yggB genes registered on the database, amino acid sequences that were highly identical to the amino acid sequence of the yggB gene of the strain with the configuration using the 19-type mutation before the mutation (SEQ ID NO: 6), by using general search software (Blast) that can be used via the internet, and the amino acid sequence of the yggB gene of *Corynebacterium callunae* (DSM20147 strain) that was registered as mentioned in (C) above is indicated as one of the highly identical sequences (70% identical at 90% coverage), following a YggB gene derived from *Corynebacterium glutamicum* (including *Brevibacterium flavum* that is classified as *Corynebacterium glutamicum* under the current classification). Moreover, when the amino acid sequence of SEQ ID NO: 6 and the amino acid sequence of the yggB gene of *Corynebacterium callunae* (DSM20147 strain) were compared by using the abovementioned software or other general software (ClustalW) that is accessible via the internet, it is found that alanine at position 100 in SEQ ID NO: 6 could be confirmed to correspond to alanine at position 98 of the DSM20147 strain.

(E) Whether or not there was difficulty, etc. in introducing a mutant-type yggB gene

derived from *Corynebacterium callunae*

According to the evidence (Exhibits Ko 54 and 92) and the entire import of oral arguments, it is found that, as of July 2016, when production by Defendant's Production Process 4 was started, if the amino acid sequence of the yggB gene of *Corynebacterium callunae* (DSM20147 strain) and the position of the amino acid into which a mutation is to be introduced were specified, a person skilled in the art would have been able to introduce the A98T mutation into that amino acid sequence and further introduce it into *Corynebacterium glutamicum* by, for example, using the process described in Description 2, and hence it cannot be found that there was technical difficulty with regard to that point.

In addition, according to the evidence (Exhibits Ko 75 through 77) and the entire import of oral arguments, it is found to have been known from before Priority Date 2 that when a gene derived from a certain bacterium is introduced into a bacterium of another species having a similar gene, the bacterium will function in a similar manner. Therefore, it cannot be said that a person skilled in the art would think introduction of a mutant-type yggB gene derived from *Corynebacterium callunae* into *Corynebacterium glutamicum* would not produce an effect, only on a basis that the bacterial species are different, and it also cannot be said that a person skilled in the art had knowledge suggesting that such configuration should be avoided.

(F) Examination

As examined in (A) through (E) above, the following can be found: Description 2 mentioned *Corynebacterium callunae* as an example of the coryneform bacterium of Invention 2 ((A) above); it was common general technical knowledge that *Corynebacterium callunae* is a glutamic acid-producing bacterium and is closely related to *Corynebacterium glutamicum* ((B) above); as the genomic analysis of *Corynebacterium callunae* was completed by March 2013 and it became possible to search or analyze the amino acid sequence of its yggB gene by using general software, it became possible to check that the amino acid sequence of the yggB gene of SEQ ID NO: 6 and that of the yggB gene of *Corynebacterium callunae* (DSM20147 strain) are highly identical, and that alanine at position 100 of SEQ ID NO: 6 corresponds to alanine at position 98 of the DSM20147 strain ((C) and (D) above); and if the position of the amino acid into which a mutation is to be introduced was specified, it would not have been technically difficult for a person skilled in the art to introduce the A98T mutation into the amino acid sequence of the DSM20147 strain and further introduce it into *Corynebacterium glutamicum*, and it also cannot be said that a person skilled in the art had knowledge suggesting that use of a mutant-type yggB gene derived from a different bacterial species should be avoided ((E) above). In light of these findings, it is reasonable

to find that, as of July 2016, when production by Defendant's Production Process 4 was started, a person skilled in the art could have easily conceived of substituting the configuration using the 19-type mutation with the configurations relating to Differences 1 and 2, in other words, replacing *Corynebacterium glutamicum* used as the strain from which the mutant-type yggB gene is derived with *Corynebacterium callunae*, and accordingly changing the position of the alanine to be substituted with threonine in the amino acid sequence of the yggB gene from position 100 to position 98.

The Defendant alleges that the ease in conceiving of the invention as mentioned in the third requirement should be construed to be the level of ease for any person skilled in the art to be able to recognize the invention in the same manner as that specified in the claims, and that such ease did not exist in this case. However, in light of the abovementioned circumstances, it should be said that the ease in conceiving of Differences 1 and 2 can be found by deeming an average technician at a glutamic acid fermentation plant using bacteria to which Invention 2 belongs, who is a person skilled in the art, as a standard, and the Defendant's allegation regarding this point cannot be adopted. In addition, while the Defendant also alleges that the priority order of intentionally selecting a yggB gene derived from *Corynebacterium callunae* in place of a yggB gene derived from *Corynebacterium glutamicum* was low, this allegation cannot be considered to overturn the abovementioned conclusion in light of the matters mentioned in Description 2 as discussed in (A) and (B) above, common general technical knowledge on *Corynebacterium callunae*, and the available analysis results mentioned in (D) above.

B. Regarding Difference 3

(A) As mentioned in (6) D. (B) above, Description 2 (paragraph [0078]) and Claim 6 (f) of Invention 2 indicated that the problem can be solved in the same manner even if a conservative substitution, such as a substitution of valine with isoleucine, is made to the amino acid sequence of the yggB gene of the strain with the configuration using the 19-type mutation (SEQ ID NO: 22).

(B) In addition, as mentioned in (6) D. (A) above, while valine at position 241 in a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) corresponds to valine at position 243 in the yggB gene that was introduced into the strain with the configuration using the 19-type mutation (the amino acid sequence of SEQ ID NO: 6 before the mutation), according to the evidence (Exhibit Ko 54, Exhibits Otsu 85 and 98) and the entire import of oral arguments, it is found that, in the same manner as in A. (D) above, a person skilled in the art could check the mutual correspondence between the abovementioned valines by using general software as of 2013, when the yggB gene of *Corynebacterium callunae* was registered on the database, and could also recognize that

the positions of these valines do not correspond to those of amino acids in the transmembrane regions disclosed in Description 2 (amino acid numbers 1–23, 25–47, 62–84, 86–108, and 110–132 of SEQ ID NO: 6; [paragraph 0073]) and the C-terminal side region (amino acid numbers 419–533; SEQ ID NO: 6; [paragraph 0070]).

(C) Moreover, according to the evidence (Exhibit Ko 54) and the entire import of oral arguments, in the same manner as in A. (E) above, if the amino acid sequence of the yggB gene of *Corynebacterium callunae* (DSM20147 strain) and the position of the amino acid into which a mutation is to be introduced were specified, it would have been possible as of July 2016, when production by Defendant's Production Process 4 was started, for a person skilled in the art to introduce the V241I mutation along with the A98T mutation into that amino acid sequence by, for example, using the process described in Description 2, and it cannot be found that there was technical difficulty with regard to that point.

(D) Accordingly, as adopting the configuration relating to Difference 3 in addition to those relating to Differences 1 and 2 for the configuration using the 19-type mutation, in other words, introducing the V241I mutation, in addition to the A98T mutation, into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain), can be considered as introducing a mutation corresponding to the conservative substitution whose introduction into the yggB gene of the strain with the configuration using the 19-type mutation was indicated in Description 2 and Claim 6 (f) as being able to solve the problem in the same manner, it is reasonable to find that a person skilled in the art could have easily conceived of the adoption of the relevant configurations as of July 2016, when production by Defendant's Production Process 4 was started, which was after the yggB gene of *Corynebacterium callunae* was registered on the database.

(E) The Defendant alleges that, in Defendant's Production Process 4, the glutamic acid-producing ability increases only when the V241I mutation is added to the A98T mutation, so it is necessary to combine these two mutations in Defendant's Production Process 4, but nothing suggested such combination, and hence it was not easy for a person skilled in the art to adopt the configuration relating to Difference 3 in addition to those relating to Differences 1 and 2. However, as mentioned in (4) C. above, it cannot be said that the glutamic acid-producing ability increases only when the V241I mutation is added to the A98T mutation, and the V241I mutation is found to be not contributing to enhancement of the glutamic acid-producing ability under a condition with an excess of biotin, the Defendant's allegation cannot be adopted.

Furthermore, the Defendant also alleges that, because even a substitution of an amino acid outside the transmembrane regions could affect efflux of glutamic acid, it could not be said that the V241I mutation clearly had no effect on efflux of glutamic acid. With

regard to this point, an article published after Priority Date 2, in 2013, as indicated by the Defendant (*Biosci. Biotechnol. Biochem.* (2013) 77 (5) 1008–1013; Exhibit Ko 31, Exhibit Otsu 87) describes that the yggB gene of *Corynebacterium glutamicum* affects glutamic acid production as a result of deleting the region of amino acid numbers 221–232 in the amino acid sequence and deleting the region of amino acid numbers 420–533, included in the C-terminal side region referred to in Description 2. However, even if such knowledge existed as of July 2016, given that the deletions at these sites differ from the V241I mutation in terms of their positions and contents of the mutation, it cannot be said that a person skilled in the art would have considered that introduction of the V241I mutation would produce a different effect from the conservative substitution indicated in Description 2 even by taking such knowledge into consideration. Thus, this point is not sufficient either to overturn the conclusion mentioned in (D) above.

C. Consequently, as it can be found that a person skilled in the art could have easily conceived of replacing the configuration using the 19-type mutation with the configurations relating to Differences 1 through 3 as of July 2016, when production by Defendant's Production Process 4 was started, Defendant's Production Process 4 using Strains [xii] and [xiii] fulfills the third requirement of the doctrine of equivalents.

(9) Regarding the fourth requirement (whether the subject process can be easily presumptively conceived of)

With regard to Defendant's Production Process 4, there are no allegations and proof on existence of circumstances that fall under the fourth requirement based on which application of the doctrine of equivalents should be denied.

(10) Regarding the fifth requirement (special circumstances)

A. Statements of the initial claims, etc. at the time of the filing and the status of corrections

According to the evidence (Exhibits Ko 79 through 82, Exhibits Otsu 80 through 84) and the entire import of oral arguments, initial Claim 1 at the time of filing the application for Invention 2 read "a coryneform bacterium having an L-glutamic acid-producing ability, wherein the coryneform bacterium has an enhanced L-glutamic acid-producing ability as compared to a non-modified strain as a result of being modified by using the yggB gene" (Exhibit Otsu 80), and Invention 2 included claims that did not specify the strain from which the mutant-type yggB gene is derived or the specific amino acid sequence before and after the mutation, but subsequently, as a result of making corrections twice in response to a notice of grounds for rejection, the statement of Claim 1 at the time of registration read as described in [Claim 1] of Attachment 5-1 "Claims (Patent 2)," and the numbers of the amino acid sequences of the mutant-type yggB gene before the mutation were specified as SEQ ID NOs: 6, 62, 68, 84, and 85 derived from

Corynebacterium glutamicum (including *Brevibacterium flavum*) or *Corynebacterium melassecola*, while the contents of the mutation to be introduced therein were also specified. As a result, it is found that a configuration that uses a mutant-type yggB gene derived from *Corynebacterium callunae*, such as in Defendant's Production Process 4, ceased to be literally included within the technical scope of Invention 2.

In addition, according to the evidence (Exhibits Ko 81 and 82, Exhibits Otsu 82 through 84), it is found that, in Description 2 at the time of the filing, *Corynebacterium callunae* was only mentioned in paragraphs [0012] and [0013], and these parts were not subject to corrections at the time of making the abovementioned corrections.

B. Determination on whether or not there are special circumstances

(A) Under the fifth requirement, application of the doctrine of equivalents cannot be asserted if there are special circumstances, such as the fact that the subject product, etc. falls under those that are intentionally excluded from the claims in the patent application procedures for the patented invention. The reason is that, for technology which the patentee had once acknowledged not to fall within the technical scope of the patented invention, or in relation to which the patentee had behaved as if he/she had objectively so acknowledged, the patentee is not entitled to make an assertion otherwise afterwards, since this is against the doctrine of estoppel (see the 1998 Supreme Court Judgment and the 2017 Supreme Court Judgment).

(B) As shown in the process of the application filing and corrections mentioned in A. above, Claim 1 at the time of the filing could comprise a configuration that uses a mutant-type yggB gene derived from *Corynebacterium callunae*, such as in Defendant's Production Process 4, but such configuration ceased to be literally comprised in Invention 2 as a result of the corrections.

(C) However, as mentioned in (8) A. (C) above, the entire genome of the *Corynebacterium callunae* DSM20147 strain and the amino acid sequence of the yggB gene were analyzed and became available in March 2013, and as of December 28, 2004, which is Priority Date 2, or as of December 28, 2005, which is the filing date of Patent 2, it was not possible to specify the amino acid sequence of the yggB gene of *Corynebacterium callunae*. Accordingly, as mentioned in (8) A. (B) above, even by taking into account that it was known from before Priority Date 2 that *Corynebacterium callunae* is a glutamic acid-producing bacterium and is closely related to *Corynebacterium glutamicum*, it is not found to have been easily possible for the Plaintiff, which is the applicant, to specify a specific configuration using a mutant-type yggB gene derived from *Corynebacterium callunae*, which could solve the problem to be solved by Invention 2, and state it in the claims in a form that satisfies the support requirement and other

description requirements at the time of the filing of the application for Invention 2.
(D) Moreover, as mentioned in A. above, the statement of Claim 1 at the time of the filing did not specify the bacterial species any more than generally stating "a coryneform bacterium having an L-glutamic acid-producing ability," and in particular, it did not state a configuration that uses a mutant-type yggB gene derived from *Corynebacterium callunae*. In addition, Description 2 only mentioned *Corynebacterium callunae* as an example of a bacterium that could be used as the coryneform bacterium of Invention 2 (paragraphs [0012] and [0013]), and no mention is made on a configuration that uses a yggB gene derived from *Corynebacterium callunae* in Description 2 either before and after the corrections.
(E) In light of the circumstances mentioned in (C) and (D) above, it cannot be said, based on the process of the application filing and corrections referred to in (B) above, that a purport to intentionally exclude a configuration that uses a mutant-type yggB gene derived from *Corynebacterium callunae* from the claims was indicated from an objective and external viewpoint. Furthermore, no special circumstances are found, based on all evidence in this case, such as a fact that Defendant's Production Process 4 had been intentionally excluded from the claims in the course of the patent application procedure of the patented invention, in relation to the fifth requirement.

(11) Summary on the existence or non-existence of infringement under the doctrine of equivalents

According to the above, Defendant's Production Process 4 that uses Strains [xii] and [xiii] is found to satisfy the first through third requirements of the doctrine of equivalents, and as no circumstances that fall under the fourth and fifth requirements are found, it is reasonable to construe that Defendant's Production Process 4 that uses Strains [xii] and [xiii] falls within the technical scope of Invention 2-5 as an equivalent to the configuration using the 19-type mutation stated in the claims.

5. Regarding Issue 3-3 (whether or not Invention 1 violates the enablement requirement or the support requirement)

In light of the case, among grounds for invalidation of Invention 1, determination is made as to whether or not Invention 1 violates the enablement requirement and the support requirement.

(1) Statements concerning the process for producing arginine in Description 1

In addition to the statements in Attachment 12 "Statements in Description 1," Description 1 has the following statements on the process for producing arginine.

A. Means for solving problem

[0012]

The amino acid as the target substance may be any amino acid as long as the gene involved in biosynthesis and its promoter are known. Specific examples of enzymes involved in biosynthesis include...

In arginine fermentation, arginine is produced through reactions catalyzed by N-acetylglutamate synthase, N-acetylglutamate kinase, N-acetylglutamylphosphate reductase, acetylornithine aminotransferase, N-acetylornitinase, ornithine carbamyltransferase, argininosuccinate synthase, and argininosuccinase. These enzymes are found to be effective. Further, these enzymes are encoded by the respective genes of argA, argB, argC, argD, argE, argF, argG, and argH in order.

[0019]

Examples of the promoter for argininosuccinate synthase include one that has at least one kind of DNA sequence selected from the group consisting of TTGCCA, TTGCTA, and TTGTCA in the -35 region and/or has the TATAAT sequence or said sequence in which the ATAAT base is substituted with another base and which does not inhibit the promotor function in the -10 region. The present invention also provides an argininosuccinate synthase gene having the abovementioned promoter.

In addition, the present invention provides a coryneform arginine-producing bacterium having the abovementioned gene.

Amino acid can be obtained by culturing a bacterium that produces the coryneform amino acid of the present invention, preferably L-glutamic acid, in a liquid medium, having the bacterium produce and accumulate the desired amino acid, preferably L-glutamic acid, in the medium, and collecting the amino acid from the medium.

In the present invention, a normal nutrient medium containing a carbon source, a nitrogen source, inorganic salts, growth factors and the like is used as a liquid medium for culturing the abovementioned strain.

As the carbon source, glucose, fructose, sucrose, waste molasses, carbohydrates such as starch hydrolysate, alcohols such as ethanol and glycerol, and organic acids such as acetic acid are used. As the nitrogen source, ammonium sulfate, ammonium nitrate, ammonium chloride, ammonium phosphate, ammonium acetate, ammonia, peptone, meat extract, yeast extract, corn steep liquor and the like are used. When an auxotrophic mutant strain is used, it is preferable to add the required substance as a sample or a natural product containing the same.

B. Working Example 6 Introduction of a mutation into the promoter region of the arginosuccinate synthase gene of a coryneform arginine-producing bacterium

[0077]

... 1) Determination of the nucleotide sequence of the upstream region of the argG gene

In order to amplify the argG gene of *Brevibacterium flavum* by PCR, the nucleotide sequences of the upstream and downstream regions of the ORF were determined. ...

[0078]

2) Prediction of the promoter site

From the above sequences, a promoter-like sequence upstream of the ORF of the argG gene was searched using commercially available software (GENETYX). A mutation was introduced at the site with the highest score (about 120 bp upstream from the first ATG), and then the activity of the promoter was evaluated.

3) Introduction of a mutation into the promoter sequence and measurement of the activity of the mutant promoter

By performing the first PCR in the site with the highest score, using the chromosomal DNA of the AJ12092 strain as a template and using the primer for mutagenesis..., and then performing a PCR again using the same chromosomal DNA as a template, with the product of the first PCR as the 3' primer, and with ... as the 5' primer, DNA fragments in which a mutation was introduced into the target promoter portion were obtained. Next, in order to measure the activity of this mutant promoter, these DNA fragments were inserted into the SmaI site of the promoter probe vector pNEOL so as to be in the forward direction toward the reporter gene lacZ, to obtain plasmids pNEOL-1, pNEOL-2, pNEOL-3, pNEOL-4 and pNEOL-7. As a control of the activity, plasmid pNEOL-0 was constructed by similarly inserting a DNA fragment, obtained by performing PCR, using chromosomal DNA of the AJ12092 strain as a template and using ..., upstream of the lacZ gene of pNEOL.

[0079]

pNEOL-0, pNEOL-1, pNEOL-2, pNEOL-3, pNEOL-4 and pNEOL-7 were introduced into the AJ12092 strain. The plasmid was introduced by the electric pulse method (Unexamined Patent Application Publication No. 1990-207791). ...

These strains were ... β-galactosidase activity was measured.

As shown in Table 20, β-galactosidase activity was detected in AJ12092/pNEOL-0, indicating that the DNA fragment inserted upstream of the lacZ structural gene functions as a promoter. In addition, β-galactosidase activity was higher in each plasmid-introduced strain as compared to AJ12092/pNEOL-0, and it was found that transcriptional activity was enhanced as shown in Table 20 by introducing a mutation into this promoter-like sequence.

[0080]

[Table 20]

	Relative activity (AJ12092/pNEOL-0=1)

AJ12092	nd
AJ12092/pNEOL-0	1.0
AJ12092/pNEOL-1	2.8
AJ12092/pNEOL-2	2.7
AJ12092/pNEOL-3	1.8
AJ12092/pNEOL-4	0.8
AJ12092/pNEOL-7	3.0

[0081]

4) Construction of plasmids for mutagenesis

A DNA fragment obtained by performing PCR using the chromosomal DNA of the AJ12092 strain as a template and using primers 14 and 15 (SEQ ID NOs: 55 and 56) was inserted into the SmaI site of a multicloning site of cloning vector pHSG398 (produced by Takara Shuzo Co., Ltd.) to construct plasmid p0. Then, p0 was digested with restriction enzymes EcoRV and BspHI, and a DNA fragment obtained by similarly digesting pNEOL-3 and pNEOL-7 with restriction enzymes EcoRV and BspHI was ligated to obtain plasmids p3 ... and p7 ... for mutagenesis.

5) Introduction of plasmids for mutagenesis into an Arg-producing bacterium

The abovementioned plasmids were introduced into the *Brevibacterium lactofermentum* AJ12092 strain, which is an Arg-producing bacterium. The plasmid was introduced by the electric pulse method (Unexamined Patent Application Publication No. 1990-207791). Since these plasmids cannot be replicated autonomously in *Brevibacterium*, only strains in which these plasmids have been integrated into the chromosome by homologous recombination can be selected as Cm-resistant strains. The strain in which the plasmids for mutagenesis have been integrated into the chromosome was selected as a chloramphenicol resistant strain in a CM2G plate medium containing 5 μg/ml of chloramphenicol (10 g of polypeptone, 10 g of yeast extract, 5 g of glucose, 5 g of NaCl, and 15 g of agar in 1 liter of pure water; pH 7.2).

Next, a strain in which the promoter portion of the argG gene was substituted with the target mutant sequence was selected from the strains that had undergone homologous recombination again and became Cm-sensitive.

As a result, a strain in which the portion was substituted with the sequence of P3 (AJ12092-P3) and a strain in which the portion was substituted with the sequence of P7 (AJ12092-P7) were obtained.

[0082]

6) Cloning of the argG gene

Based on the nucleotide sequence determined as in 1), oligonucleotides (primers 5

and 6) having the nucleotide sequences shown in SEQ ID NOs: 46 and 47 were synthesized, and PCR was performed using the chromosomal DNA of *Brevibacterium flavum* 2247 as a template. ... The obtained DNA fragment was cloned into the SmaI site in a multiple cloning site of cloning vector pSTV29 (produced by Takara Shuzo Co., Ltd.) to prepare pSTVarG. Further, pargG was prepared in which a fragment containing a replication origin obtained by treating pSAK4 described in Working Example 1(1) with SalI was inserted into the SalI site of pSTVargG.

7) Introduction of pargG into Brev.

pargG was introduced into the *Brevibacterium lactofermentum* AJ12092 strain. The plasmid was introduced by the electric pulse method (Unexamined Patent Application Publication No. 1990-207791). ...

[0083]

8) ArgG activity of promoter mutant strains

The ArgG activity of the abovementioned two types of ArgG promoter mutant strains and the strain in which argG was amplified by plasmid (AJ12092/pargG) was measured. ... Table 21 shows the ArgG activity of the abovementioned two types of ArgG promoter mutant strains and the strain in which argG was amplified by plasmid (AJ12092/pargG). As shown in Table 21, by introducing a mutation into the promoter, ArgG activity was increased to about twice that of the parent strain in AJ12092-P3 and about three times in AJ12092-P7. In addition, ArgG activity of AJ12092/pargG was about 4.5 times that of the parent strain.

[0084]

[Table 21]

	Relative activity (AJ12092=1)
AJ12092	1.0
AJ12092-P3	2.1
AJ12092-P7	2.9
AJ12092/pargG	4.4

[0085]

9) Arg production by promoter mutant strains

ArgG promoter mutant strains were cultured in flasks. As controls, parent strains AJ12092 and AJ12092/pargG were similarly cultured. ... As shown in Table 22, the Arg yield was enhanced in the argG promoter mutant strains, and the yield was equivalent to that of the strain in which argG was amplified by plasmid. In addition, while the culture time was delayed for the plasmid-amplified strain, the culture time of both promoter mutant strains AJ12092-P3 and AJ12092-P7 was equivalent to that of the parent strains,

indicating that their Arg productivity was enhanced as compared to the plasmid-amplified strain.

[0086]

[Table 22]

	OD	Arg(g/dl)	Culture time (h)	Productivity (g/dl/h)
AJ12092	0.502	1.25	48	0.026
AJ12092-P3	0.510	1.47	48	0.031
AJ12092-P7	0.514	1.43	48	0.030
AJ12092/pargG	0.520	1.47	52	0.028

(2) Regarding violation of the enablement requirement and the support requirement by the process for producing arginine

A. Regarding Invention 1-1

(A) The claim of Invention 1-1 is as described in [Claim 1] of Attachment 4-1 "Claims (Patent 1)," and Invention 1-1 is an invention of a process for producing glutamic acid or arginine by using a coryneform bacterium into which a specific nucleotide sequence has been introduced in the -35 region and/or the -10 region of the promoter sequence of any of the following: GDH gene, CS gene, ICDH gene, PDH gene, or argininosuccinate synthase gene.

(B) Description 1 states that, regarding the problem to be solved, Invention 1 provides a process for constructing a mutant strain capable of appropriately enhancing and regulating the expression level of an objective gene and having the ability to produce amino acids in high yield by genetic recombination or mutation, without using a plasmid (paragraphs [0001] and [0006]).

However, statements in Description 1 concerning the process for producing the arginine mentioned in Invention 1-1, from among amino acids, are as described in (1) above. Description 1 has statements on the enzymes relating to fermentation of arginine and the types of genes encoding them and the fact that argininosuccinate synthase is one of the enzymes related to fermentation of arginine (paragraphs [0012] and [0019]), and also describes production of arginine that uses a coryneform arginine-producing bacterium in which a mutation has been introduced into the promoter region of an argininosuccinate synthase gene as Working Example 6 (paragraphs [0077] through [0086]). However, the GDH gene, CS gene, ICDH gene, and PDH gene are all described as genes that encode enzymes related to fermentation of glutamic acid (paragraph [0012]), and they are not described to encode enzymes related to fermentation of arginine (paragraph [0012]). Furthermore, there are no statements indicating that these genes are

related to production of arginine and no working examples of production of arginine using a bacterium in which a mutation has been introduced into the promoter region of these genes.

(C) Accordingly, among the inventions claimed in Invention 1-1, at least the process for producing arginine by using a coryneform bacterium in which a mutation has been introduced into the GDH gene, CS gene, ICDH gene, or PDH gene can be considered to fall beyond the scope in which a person skilled in the art can recognize that the abovementioned problem, that is, to construct a mutant strain having the ability to produce amino acids in high yield by genetic recombination or mutation, can be solved based on the detailed descriptions of inventions in Description 1. In addition, it cannot be found that there was common general technical knowledge based on which a person skilled in the art could solve the abovementioned problem, apart from the statements in Description 1, with regard to the process for producing arginine by using these genetic mutations.

Consequently, it should be said that the patent relating to Invention 1-1 was granted in violation of the requirement prescribed in Article 36, paragraph (6), item (i) of the Patent Act (the support requirement), and should be invalidated by a trial for patent invalidation under Article 123, paragraph (1), item (iv) of the Patent Act.

B. Regarding Inventions 1-2, 1-3, and 1-4

As described in [Claim 2] through [Claim 4] of Attachment 4-1 "Claims (Patent 1)," Inventions 1-2 and 1-3 are both inventions of a process for producing glutamic acid or arginine by using a coryneform bacterium in which a specific nucleotide sequence has been introduced in the -35 region and/or the -10 region of the promoter sequence of the GDH gene. Invention 1-4 is an invention of a process for producing glutamic acid or arginine by using a coryneform bacterium in which a specific nucleotide sequence has been introduced in the -35 region and/or the -10 region of the promoter sequence of the CS gene.

Accordingly, due to the same reason as for Invention 1-1, among these inventions, at least the process for producing arginine can be considered to have fallen beyond the scope in which a person skilled in the art could recognize that the problem, that is, to construct a mutant strain having the ability to produce amino acids in high yield by genetic recombination or mutation, can be solved based on Description 1 or the common general technical knowledge at the time.

Consequently, it should be said that the patents relating to Inventions 1-2, 1-3, and 1-4 were all granted in violation of the requirement prescribed in Article 36, paragraph (6), item (i) of the Patent Act (the support requirement) and should be invalidated by a trial for patent invalidation under Article 123, paragraph (1), item (iv) of the Patent Act.

(3) Summary on the grounds for invalidation regarding Invention 1

According to the above, the patents relating to Invention 1 should all be invalidated in a trial for patent invalidation without having to determine whether there are any other grounds for invalidation, so the defense of Article 104-3, paragraph (1) of the Patent Act is established.

Since the Plaintiff has deleted Claim 3 by Correction 1, and has not asserted re-defense of correction for Invention 1-3, the Plaintiff cannot exercise its rights against the Defendant based on Invention 1-3.

On the other hand, the Plaintiff has asserted re-defense of correction for Inventions 1-1, 1-2, and 1-4, so with regard to the other grounds for invalidation alleged with regard to Invention 1-1, 1-2, and 1-4, their grounds for invalidation will be determined based on Corrected Invention1 after the correction, along with whether the abovementioned ground for invalidation, which is violation of the support requirement, has been eliminated, when determining whether re-defense of correction can be established in 6. below.

6. Regarding Issue 5 (Whether re-defense of correction of Patent 1 can be established)

(1) Contents of Corrected Invention 1

A. Contents described in Description 1

The statements in the detailed explanation of the invention in Description 1 concerning Corrected Invention 1 are outlined as described in Attachment 12 "Statements in Description 1."

B. Outline of Corrected Invention 1

According to the claims of Corrected Invention 1 (Attachment 4-2), the statements in Description 1 referred to in A. above, and the entire import of oral arguments, the outline of Corrected Invention 1 is found to be as follows.

(A) Technical field/background art

When using genetic recombination as a process for constructing a mutant strain to be used for producing amino acid by the fermentation method, a plasmid, which can be replicated autonomously and independently from the chromosome in a cell, has mainly been used for enhancing the objective gene. However, as the level of enhancement of the objective gene is decided by the copy number of the plasmid itself, there are often cases in which the expression level becomes too high due to an excessively high copy number, leading to extremely restrained growth or a decline in the target substance-producing ability, depending on the type of the objective gene. In addition, as replication of a plasmid is often unstable, there is a problem that plasmid loss sometimes occurs (paragraphs [0002] and [0003]).

(B) Problem to be solved by Corrected Invention 1

The purpose of Corrected Invention 1 is to provide a process for constructing a mutant strain capable of appropriately enhancing and regulating the expression level of the objective gene and that has the ability to produce amino acids in high yield by genetic recombination or mutation, without using a plasmid, and to provide a glutamic acid fermentation method for increasing the yield of glutamic acid and producing glutamic acid at a lower cost, using a coryneform glutamic acid-producing bacterium (paragraph [0006]).

(C) Means for solving the problem, etc.

In order to solve the abovementioned problem, Corrected Invention 1 adopted a configuration in which a mutant of a coryneform bacterium is prepared by causing or introducing by genetic recombination a mutation to the promoter sequence of an amino acid biosynthetic gene on the chromosome of the coryneform bacterium to make the sequence closer to the consensus sequence, then that mutant is cultured, and as a result, only a mutant that produces a large amount of the target amino acid is collected (paragraph [0007]).

As for examples of enzymes involved in the biosynthesis of amino acid, enzymes such as glutamate dehydrogenase (GDH) and citrate synthetase (CS) are effective in the case of glutamic acid fermentation (paragraph [0012]). Meanwhile, the abovementioned mutation to the promoter sequence may be caused either only for the promoter sequence of one gene or for the promoter sequences of two or more genes (paragraph [0015]).

In Corrected Invention 1, it is preferable that the DNA sequence in the -35 region of the promoter of the GDH gene is at least one kind of DNA sequence selected from a group consisting of TTGTCA, etc. and/or that the DNA sequence in the -10 region of that promoter is TATAAT, etc. (paragraph [0017]).

In addition, in Corrected Invention 1, examples of the promoter of the CS gene include one that has the TTGACA sequence in the -35 region and/or the TATAAT sequence in the -10 region (paragraph [0018]).

Corrected Invention 1 has a significant industrial advantage in that the target amino acid can be obtained in high yield by introducing a mutation into the promoter region of an amino acid biosynthetic gene of a coryneform amino acid-producing bacterium and regulating the expression level of the objective gene, and that the target amino acid can be obtained in high yield in a stable manner without loss as in the case of a plasmid (paragraph [0020]). In addition, Corrected Invention 1 can give a *Corynebacterium* strain the ability to produce amino acid, particularly glutamic acid, in high yield without causing an increase in byproduct aspartic acid and alanine (paragraph [0021]).

(D) Outline of the disclosed working examples

a. Working Example 1

"ATCC13869/P6-8" is a wild-type strain of a coryneform bacterium (an ATCC13869 strain of *Brevibacterium lactofermentum*) into which a plasmid of a GDH gene, wherein the -35 region of the promoter sequence (the TGGTCA sequence in the case of an ATCC13869 strain) has been modified to the TTGTCA sequence and the -10 region of the promoter sequence (the CATAAT sequence in the case of an ATCC13869 strain) has been modified toa TATAAT sequence, has been introduced.

It is indicated that the specific activity of GDH (401.3) of "ATCC13869/p6-8" is about 4.9 times that of "ATCC13869/pGDH," which is a strain into which a plasmid of a GDH gene having the same promoter sequence as that of the parent strain (the ATCC13869 strain) (paragraphs [0022] through [0025], [Table 1]).

b. Working Example 2

"FGR2" is *Brevibacterium lactofermentum* (an AJ13029 strain) to which mutations have been added, wherein the -35 region of the promoter sequence (the TGGTCA sequence) of a GDH gene of the AJ13029 strain has been mutated to a TTGTCA sequence, and the -10 region of the promoter sequence (CATAAT sequence) has been mutated to a TATAAT sequence.

It is indicated that the specific activity of GDH of FGR2 is about 3.4 times that of an AJ13029 strain, and that its L-glutamic acid production (3.0 g/dl) increased compared to the AJ13029 strain (2.6 g/dl) (paragraphs [0026], [0029], [0031], and [0032], [Table 5], [Table 6]).

c. Working Example 3

"GB02" is a strain into which a mutation whereby the CS gene on the chromosome of FGR2 referred to in b. above is substituted with a CS gene in which the -10 region of the promoter sequence has been modified to a TATAAT sequence (the sequence before the mutation is TATAGC) has been introduced."GB03" is a strain into which a mutation whereby the CS gene on the chromosome of FGR2 is substituted with a CS gene in which the -10 region of the promoter has been modified to a TATAAT sequence and the -35 region of the promoter has been modified to a TTGACA sequence (the sequence before the mutation is ATGGCT) has been introduced. The -35 region and the -10 region of the promoter sequence of the GDH gene of GB02 and GB03 are the same as those of FGR2.

It is indicated [i] that the specific activity of citrate synthetase of GB02 is about 1.9 times that of FGR2 and the specific activity of citrate synthetase of GB03 is about 4.0 times that of FGR2, and [ii] that the L-glutamic acid production of GB02 and GB03 (9.4 g/l respectively) increased compared to FGR2 (8.9 g/l).

(Regarding the above, paragraphs [0033] through [0036], paragraphs [0041] through

[0046], paragraph [0048], [Table 7], [Table 10], [Table 12]).

d. Working Example 7

"GA02" is a strain wherein a mutation has been introduced on the chromosome of the AJ13029 strain referred to in b. above by using the promoter sequence of the GDH gene of FGR2 referred to in b. above, and the -35 region and the -10 region of the promoter sequence of the GDH gene introduced are the same as those of FGR2.

It is indicated that the specific activity of GDH of GA02 is about 3.5 times that of the parent strain (AJ13029), and that the L-glutamic acid production of GA02 (2.9 g/dl) increased compared to AJ13029 (2.6 g/dl) (paragraphs [0087] through [0089], [Table 23]).

(2) Whether or not Defendant's Production Processes 1 and 3 fall within the technical scope of Corrected Invention 1

According to the entire import of oral arguments, it is found that, when Defendant's Production Processes 1 and 3 are compared with Corrected Invention 1, the results of the comparison will be as described in Attachment 6-2 "Comparison between Defendant's Production Processes 1 and 3 and Corrected Invention 1," and therefore Defendant's Production Process 1 literally falls within the technical scopes of Corrected Invention 1-1 (corresponding to Invention 1-1), Corrected Invention 1-2 (corresponding to Invention 1-2), and Corrected Invention 1-3 (corresponding to Invention 1-4), whereas Defendant's Production Process 3 literally falls within the technical scope of Corrected Invention 1-1 but does not fall within the technical scope of Corrected Invention 1-3.

(3) Regarding Exhibit Otsu 6 Invention

A. Contents described in Exhibit Otsu 6 Document

The contents described in Exhibit Otsu 6 Document include the following (Exhibit Otsu 6, the entire import of oral arguments; for Figures 1 and 5 and Table 2, see Attachment 14 "Drawings of Cited Documents").

(A) Abstract (Lines 1 through 18 on page 1297)

"Relatively limited information about promoter structures in *Corynebacterium glutamicum* has been available until now. ... A comparative analysis of the newly characterized promoter sequences together with published promoters from *C. glutamicum* revealed conserved sequences centred about 35 bp (ttGcca) and 10 bp (TA.aaT) upstream of the TS [transcriptional start] site. The position of these motifs and the motifs themselves are comparable to the -35 and -10 promoter consensus sequences of other Gram-positive and Gram-negative bacteria, indicating that they represent transcription initiation signals in *C. glutamicum*."

(B) Figure 1 (page 1301)

"Fig. 1. Nucleotide sequences of *C. glutamicum* promoters aligned according to their

TS sites. Putative -35 and -10 region identified by the promscan program are underlined. The promoter sequences of *hom, thrC, fda, lysA, ask, gdh, glt, gap, pgk* and *trp* were taken from references cited in the text."

(C) Discussion

a. Line 11 in the right column on page 1305 through line 2 in the left column on page 1306

"All 18 fragments tested also drove transcription of *cat* in *E. coli*, indicating that the isolated promoters were active in this organism too. This result was not unexpected since many of the genes cloned from *C. glutamicum* were expressed in *E. coli* as indicated by heterologous complementation of appropriate auxotrophs ... Conversely, several *E. coli* genes were efficiently expressed in *C. glutamicum* ... and the *E. colitac, lacUV5*, and *trp* promoters have been shown to be functional in corynebacteria ... All these results indicate that the general structure of promoters from *C. glutamicum* is similar to that of promoters from *E. coli*. However, there were also reports on corynebacteria-specific promoters which were obviously not functional in *E. coli* ..."

b. Lines 3 through 38 in the left column on page 1306

"The similarity of the primary structure of promoters from *C. glutamicum* with that of promoters from other bacteria was substantiated by comparative computer analysis. Both programs applied in this study showed that in our set of *C. glutamicum* promoters the most correlated region is located around 10 bp upstream of the TS site and comprises the hexamer TA.AAT. This or a similar motif can be found in all promoters. Another conserved region was detected about 35 bp upstream of the TS site. The consensus hexamer TTGCCA found in this region differs from the *E. coli* consensus TTGACA only in the fourth position. The role of the TTGCCA motif is not that obvious since it is clearly seen (> 3 bp matching) in only 14 promoters out of the 33. In several others the motif was not easily discernible, indicating a lower conservation in *C. glutamicum* promoters.

However, from the position relative to the TS site, from the spacing (a mean of 17‘35 bp) and from the sequence of the two conserved hexamer motifs, it is obvious that these signals in *C. glutamicum* promoters are comparable to the -10 and -35 promoter consensus sequences of *E. coli* ... and other eubacteria, e.g. *Bacillus* ..., *Eactobacillus* ... and *Streptococcus* ... The significance of the *C. glutamicum* consensus motifs found by our analyses is corroborated by the fact that the *tac* promoter (11 out of 12 positions identical to consensus) was found to be very efficient in *C. glutamicum* ... and that an alteration of the -10 hexamer in the *E. coli lac* promoter from TATGTT to TATATT, thus increasing the similarity to the consensus sequence, led to higher efficiency of the promoter in *C. glutamicum* ..."

c. Lines 18 through 42 in the right column on page 1306

"In some Gram-positive bacteria, e.g. *Bacillus* and *Streptococcus*, most of the -10 and -35 promoter regions match their consensus sequences TATAAT and TTGACA, respectively, nearly perfectly ... In contrast, this does not hold for the set of *C. glutamicum* promoters studied here. Several of the bases in the *C. glutamicum* promoter consensus motifs are only moderately conserved (see Fig. 5), indicating only relatively moderate conservation of the motif as a whole. Since the conservation level of promoter consensus sequences probably reflects the requirements of the major RNA polymerase σ-factor in a given organism ..., it was interesting to compare the degree of consensus conservation per position in our study with that in compilations of promoters from other bacteria. As shown in Table 2, nearly all bases in the -35 and -10 consensus hexamers in the *Bacillus, Eactobacillus* and *Streptococcus* promoters are much more and those in the *E. coli* promoters are considerably more conserved than in the set of *C. glutamicum* promoters. These data suggest that the recognition specificity of the major RNA polymerase in the organisms listed decreases in the order *Bacillus*/*Lactobacillus*/*Streptococcus* > *E. coli* > *C. glutamicum*."

d. Table 2 (page 1307)

"Table 2. Degree of consensus conservation in the -35 and -10 motifs in compilations of promoters from different organisms

Promoter compilations for the different organisms were taken from the following references: *C. glutamicum*, 33 promoters (this work); *E. coli*, 263 promoters ...; *Bacillus subtilis*, 237 promoters ...; *Eactobacillus*, 30 promoters ...; *Streptococcus*, 17 promoters ..."

e. Line 2 from the bottom in the right column on page 1306 through line 6 in the left column on page 1307

"Since the activity of promoters in *E. coli* can be correlated to a major extent with their similarity to the -35 and -10 consensus hexamers ..., we assumed that the observed CAT activity roughly correlates with the identity score of a given promoter with the predicted consensus promoter sequence. However, such a correlation could not be recognized."

f. Lines 4 through 10 in the right column on page 1307

"It becomes clear from the data presented here that further work, e.g. stringent mutational and biochemical analysis of selected promoters, is necessary to gain detailed information on the structure-function relationship of promoters (or promoter classes) in *C. glutamicum* and thus a better understanding of gene expression in this industrially important organism."

B. Outline of Exhibit Otsu 6 Invention

As mentioned in A(B) above, Figure 1 of Exhibit Otsu 6 Document shows nucleic acid sequences of the promoters of *Corynebacterium glutamicum* genes, including the GDH gene and the CS gene. The figure indicates nucleic acid sequences of the promoters of *Corynebacterium glutamicum* genes which have a "TGGTCA" sequence in the -35 region and a "CATAAT" sequence in the -10 region of the promoter sequence of the GDH gene ("p-gdh"), and a "TGGCTA" sequence in the -35 region and a "TAGCGT" sequence in the -10 region of the promoter sequence of the CS gene ("p-glt").

Consequently, Exhibit Otsu 6 Document is found to disclose Exhibit Otsu 6 Invention having the following configuration as a configuration to be compared with Corrected Invention 1.

"A coryneform bacterium which has a TGGTCA sequence in the -35 region and a CATAAT sequence in the -10 region of the promoter sequence of the GDH gene, and a TGGCTA sequence in the -35 region and a TAGCGT DNA sequence in the -10 region of the promoter sequence of the CS gene on the chromosome of the coryneform bacterium"

C. Other matters disclosed in Exhibit Otsu 6 Document

According to the matters described in Exhibit Otsu 6 Document mentioned in A. above, Exhibit Otsu 6 Document is found to disclose the following, in addition to Exhibit Otsu 6 Invention referred to in B. above.

(A) As a result of analyzing promoters of *Corynebacterium glutamicum* genes, conserved sequences are ttGcca.a in the -35 region and ggTA.aaT in the -10 region, and the position of these motifs and the motifs themselves correspond to consensus sequences in the -35 region and the -10 region of promoters of other Gram-positive and Gram-negative bacteria (A. (A) above, Figure 5).

(B) Between *Corynebacterium glutamicum* and *E. coli*, there are examples where the promoter sequence of either is functional in the other, suggesting similarity in the general structure, etc. of promoter regions of *Corynebacterium glutamicum* genes and promoter regions of *E. coli*, but there were also multiple reports on corynebacteria-specific promoters which were obviously not functional in *E. coli* (A. (C) a., b. above).

(C) Several of the bases in the consensus motifs of *Corynebacterium glutamicum* are only moderately conserved, and the conservation levels of most bases were lower than those of Gram-positive bacteria, such as *Bacillus* and *Streptococcus*, and *E. coli* (A. (C) c., d. above, Figure 5, Table 2).

(D) Whereas the activity of promoters in *E. coli* can be correlated to a major extent with their similarity to the consensus sequences in the -35 region and the -10 region, the activity of promoters in *Corynebacterium glutamicum* was not recognized to correlate

with their similarity to the predicted consensus sequence (A. (C) e. above).

(4) Regarding Exhibit Otsu 9 Invention

A. Contents described in Exhibit Otsu 9 Document

The contents described in Exhibit Otsu 9 Document include the following (Exhibit Otsu 9; for Table 9, see Attachment 14 "Drawings of Cited Documents")

(A) Claims (Lines 2 through 8 on page 55)

"1. a coryneform L-glutamic acid-producing bacterium deficient in α-ketoglutarate dehydrogenase activity due to occurrence of substitution, deletion, insertion, addition, or inversion of one or more nucleotides in a nucleotide sequence of a gene coding for an enzyme having α-KGDH activity or a promoter thereof existing on chromosome;

2. a method of producing L-glutamic acid comprising the steps of cultivating the coryneform L-glutamic acid-producing bacterium described in Claim 1 in a liquid medium, to allow L-glutamic acid to be produced and accumulated in a culture liquid, and collecting it"

(B) Description

a. Technical field (Lines 4 through 11 on page 1)

"The present invention relates to breeding and utilization of coryneform bacteria used for fermentative production of L-glutamic acid... In particular, the present invention relates to coryneform L-glutamic acid-producing bacteria deficient in α-ketoglutarate dehydrogenase (α-KGDH), a method of producing L-glutamic acid by using the bacteria..."

b. Background art (Line 13 on page 1 through line 5 on page 2)

"L-Glutamic acid has been hitherto industrially produced by a fermentative method using coryneform bacteria belonging to the genus *Brevibacterium* or *Corynebacterium*.

Recently, it has been revealed that a mutant strain of *Escherichia coli,* in which the α-KGDH activity is deficient or lowered, and the glutamic acid-decomposing activity is lowered, has high L-glutamic acid productivity...

On the contrary, it was reported that a mutant strain having lowered α-KGDH activity had approximately the same L-glutamic acid productivity as that of its parent strain in the case of a bacterium belonging to the genus *Brevibacterium*... Therefore, it has been believed that the level of α-KGDH activity is not important for production of L-glutamic acid in coryneform bacteria.

On the other hand, it was found that a mutant strain of a L-glutamic acid-producing bacterium belonging to the genus *Brevibacterium* having lowered α-KGDH activity produces L-glutamic acid at high efficiency ... when the bacterium is cultivated in a medium which contains a material containing an excessive amount of biotin as a carbon

source without addition of materials which suppress an effect of biotin such as penicillins and surface-active agents... However, since it has been believed that the level of α-KGDH activity is not important for production of L-glutamic acid in the coryneform bacteria as described above, there has been no example in which an α-KGDH gene of a coryneform L-glutamic acid-producing bacterium is cloned and analyzed. Further, mutant strains of coryneform bacteria being completely deficient in α-KGDH have been unknown."

c. Disclosure of the invention

(a) "An object of the present invention is to obtain an α-KGDH gene originating from coryneform L-glutamic acid-producing bacteria, prepare recombinant DNA containing the gene, clarify the influence of the level of α-KGDH activity on fermentative production of L-glutamic acid by using microorganisms transformed with the recombinant DNA, and thus provide a new methodology in breeding of coryneform L-glutamic acid-producing bacteria. More specifically, an object of the present invention is to obtain a coryneform L-glutamic acid-producing bacterium deficient in α-KGDH activity by destroying an α-KGDH gene existing on chromosomal DNA, and provide a method of producing L-glutamic acid by using the bacterium. ...

The present inventors have obtained an α-KGDH gene originating from a coryneform L-glutamic acid-producing bacterium, clarified its structure, transformed a coryneform L-glutamic acid-producing bacterium by using a plasmid into which the gene is incorporated, and investigated the level of α-KGDH activity and L-glutamic acid productivity of obtained transformants. As a result, it has been found that the α-KGDH activity remarkably affects production of L-glutamic acid. Further, the present inventors have found that a strain, in which the α-KGDH activity is deleted by destroying an α-KGDH gene existing on chromosome of a coryneform L-glutamic acid-producing bacterium, produces and accumulates a considerable amount of L-glutamic acid when it is cultivated in a medium containing an excessive amount of biotin without adding any substance for suppressing the action of biotin such as surfactant and penicillin." (Lines 7 through 27 on page 2)

(b) "Namely, the present invention provides:

(1) a coryneform L-glutamic acid-producing bacterium deficient in α-KGDH activity due to occurrence of substitution, deletion, insertion, addition, or inversion of one or more nucleotides in a nucleotide sequence of a gene coding for an enzyme having α-KGDH activity or a promoter thereof existing on chromosome;

(2) a method of producing L-glutamic acid comprising the steps of cultivating the coryneform L-glutamic acid-producing bacterium described in the aforementioned item (1) in a liquid medium, to allow L-glutamic acid to be produced and accumulated in a

culture liquid, and collecting it; ..." (Lines 3 through 18 on page 3)

(c) "Utilization of the gene of the present invention includes preparation of α-KGDH activity-deficient strains by insertion of a drug-relevant gene or the like, preparation of strains with weak activity *in vitro* mutation, preparation of expression lowered strains by modification of a promoter and so on, which makes it possible to efficiently breed a bacterial strain in which the L-glutamic acid productivity is further improved as compared with conventional coryneform L-glutamic acid-producing bacteria.

A strain deficient in α-KGDH activity can be obtained either by a method which uses a chemical reagent to induce mutation, or by a method which resides in genetic recombination. However, in the case of the method for introducing mutation by using a chemical reagent, it is relatively easy to obtain a strain in which the α-KGDH activity is lowered, but it is difficult to obtain a strain in which the activity is completely deficient. In order to obtain the latter strain, it is advantageous to use a method in which an α-KGDH gene existing on chromosome is modified or destroyed by means of a genetic homologous recombination method on the basis of the structure of the α-KGDH gene having been clarified as described above.

...

Specifically, substitution, deletion, insertion, addition or inversion of one or a plurality of nucleotides is caused in a nucleotide sequence in a coding region or a promoter region of the α-KGDH gene by means of a site-directed mutagenesis method ... or a treatment with a chemical reagent such as sodium hyposulfite and hydroxylamine ... The gene thus modified or destroyed is used to substitute a normal gene on chromosome. It is thereby possible to delete the activity of α-KGDH as a gene product, or extinguish transcription of the α-KGDH gene." (Line 20 on page 8 through line 10 on page 9)

"The method for substituting a normal gene on chromosome of a coryneform L-glutamic acid-producing bacterium with the gene thus obtained by introduction of mutation to give modification or destruction includes a method which utilizes homologous recombination ... Selection of such bacterial strains makes it possible to obtain a bacterial strain in which a normal gene on the chromosome is substituted with a gene into which nucleotide substitution, deletion, insertion, addition or inversion is introduced to provide modification or destruction." (Line 29 on page 9 through line 13 on page 10)

"A coryneform L-glutamic acid-producing bacterium deficient in α-KGDH activity thus obtained is remarkably more excellent in L-glutamic acid productivity especially in a medium containing an excessive amount of biotin than strains having partially lowered α-KGDH activity." (Lines 14 through 16 on page 10)

(d) "In order to improve the L-glutamic acid productivity, it is advantageous to enhance glutamic acid biosynthetic genes. Examples of enhancement of the glutamic acid biosynthesis system genes include phosphofructokinase in the glycolytic pathway (PFK ...), phosphoenolpyruvate carboxylase in the anaplerotic pathway (PEPC ...), citrate synthase in the TCA cycle (CS ...), aconitate hydratase (ACO ...), isocitrate dehydrogenase (ICDH ...), glutamate dehydrogenase for amination reaction (GDH ...), and so on." (Lines 11 through 20 on page 11)

"In order to obtain the genes described above, the following methods may be available.

(1) As a mutant strain in which mutation arises in an objective gene and a characteristic character is presented, a mutant strain is obtained wherein the character disappears by introducing the objective gene. A gene which complements the character of the mutant strain is obtained from chromosome of a coryneform bacterium.

(2) When an objective gene has been already obtained from another organism, and its nucleotide sequence has been clarified, the objective gene is obtained by a technique of hybridization using DNA in a region having high homology as a probe.

(3) When a nucleotide sequence of an objective gene is fairly clarified in detail, a gene fragment containing the objective gene is obtained by means of a PCR method ... using chromosome of a coryneform bacterium as a template.

...

When the gene is obtained in accordance with the methods of (2) and (3) described above, if an objective gene has no original promoter, the objective gene can be expressed by inserting a DNA fragment having promoter activity in coryneform bacteria into a position upstream from the objective gene. In order to enhance expression of the objective gene, it may be available to ligate the objective gene at a position downstream from a strong promoter. Strong promoters, which function in cells of coryneform bacteria, include *lac* promoter, *tac* promoter, *trp* promoter, etc. from *Escherichia coli* ... In addition, *trp* promoter from a bacterium belonging to the genus *Corynebacterium* is also a preferable promoter ... In Examples of the present invention, *trp* promoter from a coryneform bacterium has been used for expression of the PEPC gene." (Line 21 on page 11 through line 16 on page 12)

d. Description of preferred embodiments

(a) "Example 3: Preparation of α-KGDH Gene-Deficient Strain

According to the fact that the production of L-glutamic acid was suppressed by amplification of the α-KGDH gene, it was expected, on the contrary, that the yield of glutamic acid could be improved by destroying the α-KGDH gene. A gene-destroyed strain was obtained by a homologous recombination method using a temperature-

sensitive plasmid described in Japanese Patent Laid-open No. 1993-7491." (Lines 18 through 22 on page 23)

"A nucleotide sequence of the α-KGDH gene on chromosome was investigated by using the sensitive strain, and it was confirmed that the α-KGDH gene was substituted into the deficient type. The strain was designated as ΔS strain." (Lines 21 through 23 on page 24)

(b) "Example 4: Preparation of Plasmids for Amplifying *gdh*, *glt*A and *icd* Genes" (Line 26 on page 24)

"Next, a promoter of tryptophan operon known as a promoter to function in *Brevibacterium lactofermentum* (...) was inserted at a position upstream from the *ppc* gene on pHSG-ppc'. ... Thus a plasmid pHSG-ppc, in which one copy of the promoter of the tryptophan operon was inserted at a position upstream from the *ppc* gene, was obtained." (Lines 2 through 15 on page 26)

(c) "Example 7: Confirmation of Expression of Each Gene on pGDH, pGLTA, pPPC, pICD, pGDH+GLTA+ICD, and pGDH+GLTA+PPC

It was confirmed whether or not each of the genes on pGDH, pGLTA, pPPC, pICD, pGDH+GLTA+ICD, and pGDH+GLTA+PPC was expressed in cells of *Brevibacterium lactofermentum*, and these plasmids functioned for gene amplification. Specifically, each of the plasmids was introduced into *Brevibacterium lactofermentum* ATCC13869 by means of an electric pulse method (...)." (Lines 3 through 8 on page 28)

(d) "Example 8: L-glutamic Acid Production by ΔS Strain, and ΔS Strains with Amplified *gdh*, *glt*A, *ppc* and *icd* Genes"(Lines 13 through 14 on page 30)

"(1) ... It was confirmed that the ΔS strain produced and accumulated L-glutamic acid at a high yield although it was cultivated in the medium containing an excessive amount of biotin." (Lines 10 and 11 on page 31)

"(2) ... pGDH, pGLTA, pPPC, pICD, pGDH+GLTA+ICD, or pGDH+GLTA+PPC prepared as described above was introduced into the ΔS strain to evaluate L-glutamic acid productivity of transformants in which each of the plasmids was introduced. Introduction of the plasmids into cells of *Brevibacterium lactofermentum* was performed in accordance with an electric pulse method (...)." (Lines 12 through 18 on page 31)

"Evaluation of L-glutamic acid productivity of the ΔS strain and the obtained transformants was performed as described in the aforementioned item (1). ... Results are shown in Table 9." (Lines 22 through 24 on page 31)

e. Industrial applicability

"It has been revealed that the level of α-KGDH activity of coryneform L-glutamic acid-producing bacteria affects fermentative production of L-glutamic acid. Therefore, it

becomes possible to efficiently breed bacterial strains having further improved L-glutamic acid productivity as compared with conventional coryneform L-glutamic acid-producing bacteria, by preparing α-KGDH gene activity-deficient strains by insertion of drug-relevant genes and so on, by preparing activity-leaky strains by *in vitro* mutation, and by preparing strains with lowered expression by modification of promoters and so on." (Lines 13 through 18 on page 33)

B. Outline of Exhibit Otsu 9 Invention

(A) According to A. above, Exhibit Otsu 9 Document is found to disclose the following.

a. In a coryneform L-glutamic acid-producing bacterium, the activity of the α-KGDH gene remarkably affects production of L-glutamic acid, and a coryneform L-glutamic acid-producing bacterium in which the α-KGDH activity is deleted produces and accumulates a considerable amount of L-glutamic acid (A. (B) c. (a) above). One method of deleting the activity of the α-KGDH gene is to modify or destroy the α-KGDH gene by causing occurrence of substitution, deletion, insertion, addition, or inversion of one or more nucleotides in a nucleotide sequence of a gene coding for an enzyme having α-KGDH activity or a promoter thereof existing on the chromosome (A. (B) c. (b), (c) above).

b. Besides deletion of the activity of the α-KGDH gene, there is a method that improves the glutamic acid productivity by enhancing the glutamic acid biosynthetic genes. As a method to enhance genes, it may be available to ligate the objective gene for the purpose of enhancing expression of the objective gene. Strong promoters, which function in cells of coryneform bacteria, include *lac* promoter, *tac* promoter, *trp* promoter, etc. from *Escherichia coli*, and *trp* promoter from a bacterium belonging to the genus *Corynebacterium* (A. (B) c. (d) above).

c. A working example in which the activity of the α-KGDH gene on the chromosome of a coryneform bacterium is deleted, and then glutamic acid biosynthetic genes, such as the GDH gene and the CS gene (*glt*A), are enhanced by introducing plasmids (vectors) containing these genes (A. (B) d. above).

(B) Exhibit Otsu 9 Invention

According to (A) above, Exhibit Otsu 9 Document is found to disclose Exhibit Otsu 9 Invention having the following configuration as a configuration to be compared with Corrected Invention 1.

"A method of producing L-glutamic acid by the fermentation method comprising the steps of cultivating, in a medium, a coryneform bacterium, which is deficient in α-ketoglutarate dehydrogenase activity due to occurrence of substitution, deletion, insertion, addition, or inversion of one or more nucleotides in a nucleotide sequence of a gene

coding for an enzyme having α-KGDH activity or a promoter thereof existing on the chromosome, and into which a vector wherein a glutamic acid biosynthetic gene, such as the GDH gene or the CS gene, is ligated at a position downstream from a strong promoter has been introduced, to allow L-glutamic acid to be produced and accumulated in the medium, and collecting it from the medium."

(C) Regarding the Defendant's allegations

The Defendant alleges that Exhibit Otsu 9 Document discloses an invention of "a method of producing L-glutamic acid by the fermentation method comprising the steps of cultivating, in a medium, a coryneform bacterium in which a mutation has been introduced into the promoter sequence of the glutamate dehydrogenase (GDH) gene or citrate synthetase (CS) gene on the chromosome of the coryneform bacterium, to allow L-glutamic acid to be produced and accumulated in the medium, and collecting it from the medium."

As mentioned in A. (B) c. (c) above, Exhibit Otsu 9 Document describes a method of introducing a mutation into a gene or the promoter sequence thereof on a chromosome by utilizing homologous recombination, but this description has been described as a means for causing defects in the activity of the α-KGDH gene, and not as a means for enhancing the GDH gene, etc. on a chromosome. In addition, as mentioned in (A) b. above, Exhibit Otsu 9 Document discloses a method of ligating the objective gene at a position downstream from a strong promoter as a method to enhance glutamic acid biosynthetic genes. However, the only specific method for enhancing the GDH gene or the CS gene described in Exhibit Otsu 9 Document is the method of introducing vectors (plasmids) of these genes referred to in A. (B) d. above, and it does not describe a method of modifying the promoter sequence of the GDH gene or the CS gene on a chromosome.

In this way, it cannot be said that Exhibit Otsu 9 Document discloses introduction of a mutation into the promoter sequence of the GDH gene or the CS gene on the chromosome of a coryneform bacterium. Therefore, the Defendant's abovementioned allegation cannot be adopted, and it is reasonable to find Exhibit Otsu 9 Invention to be as mentioned in (B) above.

(5) Regarding matters described in publicly known documents other than Exhibit Otsu 6 Document and Exhibit Otsu 9 Document

A. Regarding Exhibit Otsu 8 Document

(A) Matters described in Exhibit Otsu 8 Document

The contents described in Exhibit Otsu 8 Document include the following (Exhibit Otsu 8, Exhibit Ko 25).

a. Introduction

"Citrate synthase (EC4.1.3.7) catalyses a crucial step at the entry of the citric acid cycle, i.e. the condensation of acetyl-CoA and oxaloacetate to form citrate and CoA. The key position of this enzyme within central metabolism has given rise to much interest in its structural, kinetic, regulatory and molecular characteristics and, therefore, it has been studied in great detail from a variety of different organisms (...)." (Lines 1 through 9 in the left column on page 1818)

"The same authors reported that a classically obtained mutant of *C. glutamicum* ssp. *flavum* with reduced citrate synthase activity was able to produce significant amounts of aspartate and lysine (...). This and the key position of the enzyme in the carbon flow from carbohydrates to the citric acid cycle, and thus also to the amino acids derived therefrom, suggest that citrate synthase may be an important target in the genetic construction of defined amino-acid-producing *C. glutamicum* strains.

We describe here the citrate synthase of *C. glutamicum* with respect to its regulation, the isolation of the citrate synthase gene (*gltA*), its nucleotide sequence, the homologous and heterologous expression and its transcriptional organization." (Lines 27 through 40 in the left column on page 1818)

b. Results and discussion

(a) "It is noteworthy that the *gltA*-overexpressing *C. glutamicum* strains showed slower growth on all media tested (e.g. on LB medium a doubling time of 120 min instead of 80 min), indicating a slight impairment of the cells by the enhanced level of citrate synthase." (Lines 32 through 36 in the right column on page 1820)

(b) "To test the effect of increased citrate synthase activity on glutamate secretion, standard glutamate fermentations (Hoischen & Krämer, 1989) were performed with *C. glutamicum* WT and WT (pJC-gltA3A). In these experiments identical glutamate secretion rates of about 17 μmol min^{-1} (g dry weight)$^{-1}$ were found for both strains. Thus, the capacity of *C. glutamicum* to secrete glutamate cannot be enhanced by simply elevating the citrate synthase enzyme level." (Lines 17 through 26 in the left column on page 1821)

(c) "The nucleotide sequence obtained and the deduced amino acid sequence of the *C. glutamicum* citrate synthase are shown in Fig. 2." (Lines 10 through 12 in the right column on page 1821)

"Fig. 2. Nucleotide sequence of the 3007 bp SalI-HindIII fragment and the deduced citrate synthase amino acid sequence. The transcriptional initiation site (↦), the putative -10 region (—) ... are shown." (Figure 2 on pages 1822 through 1823; a "TATAGC" sequence is shown in the putative -10 region in Figure 2)

(B) According to the matters described in Exhibit Otsu 8 Document mentioned in (A)

above and the entire import of oral arguments, Exhibit Otsu 8 Document discloses the following: citrate synthetase (CS) catalyzes a crucial reaction of the citric acid cycle; the production of aspartate and lysine increased in *Corynebacterium glutamicum* with reduced CS activity; overexpression of CS slows the growth of *Corynebacterium glutamicum*; and in *Corynebacterium glutamicum* in which CS activity was increased by introducing plasmids, the glutamic acid productivity was not improved compared to a strain to which no mutation was added. It also discloses that the sequence in the -10 region of the promoter of the CS gene of wild-type *Corynebacterium glutamicum* was deduced as being TATAGC.

B. Regarding Exhibit Otsu 10 Document

(A) Matters described in Exhibit Otsu 10 Document

Exhibit Otsu 10 Document has the following description concerning the promoter sequence of *E. coli* (Exhibit Otsu 10).

"Six mutants with a 21-fold increase in promoter strength compared with the wild-type were mutated in the -35 promoter region from TTGTCA to the consensus sequence TTGACA. The -10 region sequence TACAAT was mutated to the consensus sequence TATAAT in three mutants exhibiting an ampC promoter seven times stronger than the wild-type." (Lines 4 through 10 of Abstract in the left column on page 875)

"The very conserved TTG sequence of the -35 region is followed downstream by three less stringently conserved nucleotides." (Lines 18 through 20 of Introduction in the left column on page 875)

(B) According to the matters described in Exhibit Otsu 10 Document mentioned in (A) above, Exhibit Otsu 10 Document discloses that, in *E. coli*, mutants in which the TTGTCA sequence in the -35 region was mutated to the consensus sequence TTGACA showed a 21-times increase in promoter strength, that mutants in which the TACAAT sequence in the -10 region was mutated to the consensus sequence TATAAT showed a seven-time increase in promoter strength, and that the TTG sequence is conserved in the -35 region.

C. Regarding Exhibit Otsu 11 Document

Exhibit Otsu 11 Document discloses that, in *E. coli*, the -35 region and the -10 region of the *tac* promoter sequence are TTGACA and TATAAT, respectively, and the -35 region and the -10 region of the *lac*UV5 promoter are TTTACA and TATAAT, respectively (Fig. 1*b* on page 3540) (Exhibit Otsu 11, the entire import of oral arguments).

D. Regarding Exhibit Otsu 12 Document

(A) Matters described in Exhibit Otsu 12 Document

The contents described in Exhibit Otsu 12 Document include the following (Exhibit

Otsu 12, the entire import of oral arguments).

a. Claims

"1. A DNA fragment (c) having a nucleotide sequence (a) represented by TAGACA and a nucleotide sequence (b) represented by TATAAT at a position 15 to 20 nucleotide pairs downstream of said nucleotide sequence (a), and capable of functioning as a promoter in a coryneform bacterium cell." (Lines 5 through 9 in the left column on page 1)

b. Detailed explanation of the invention

(a) Working Example 2

"Introduction of a synthetic promoter into pPR3:

The promoter was synthesized by using a DNA synthesizer ... (making it a fragment with *Bam*HI ends at both ends). The nucleotide sequence of that DNA fragment is shown below." (Lines 13 through 18 in the upper right column on page 6; for the cited nucleotide sequence, see Attachment 14 "Drawings of Cited Documents")

"By reacting 0.5 μg of pPR3, the plasmid prepared in Working Example 1, with restriction enzyme *Bam*HI (5 units) at 37°C for one hour, the plasmid DNA was completely degraded.

The abovementioned synthetic promoter DNA and the plasmid DNA degradation product were mixed, ... *E. coli* HB101 Competent Cells (produced by Takara Shuzo) were transformed by using this solution.

The transformed strains were cultured in ..., and were grown. The plasmids of these grown strains were extracted by the alkaline SDS method ...

The obtained plasmids were transformed into plasmid-cured *Brevibacterium flavum* MJ-233 strains (FERM BP-1497) according to the method described in item (E) of Working Example 1, and a plasmid was extracted by using the method described in item (A) of Working Example 1.

It was confirmed that the synthetic DNA was integrated into pPR3 based on the cleavage patterns of restriction enzymes, including *Bam*HI, *Kpn*I, and *Sac*I, and this plasmid was named "pPR3BT2." (Line 1 in the lower left column through line 11 in the lower right column on page 6.)

(b) Working Example 3

"Measurement of the strength of the synthetic promoter:

The strength of the promoter inserted into pPR3 in Working Example 2 was checked by measuring the activity of chloramphenicol acetyltransferase (CAT).

A *Brevibacterium flavum* MJ-233 strain carrying pPR3 and a *Brevibacterium flavum* MJ-233 strain carrying pPR3BT2 were each precultured overnight in a test tube

containing 10 ml medium prepared by adding 50 μg/ml kanamycin to semisynthetic medium A described in item (A) of Working Example 1. Then, the culture solution was cultured for about six hours in a conical flask containing 100 ml of the abovementioned medium, after which bacterial cells were harvested and used for activity measurement. The CAT activity was measured by a method of W. V. Shaw et al. ... As a result, the MJ-233 strain carrying pPR3BT2 had about 14 times higher CAT activity compared to the MJ-233 strain carrying pPR3 without the insertion of the promoter." (Line 12 in the lower right column on page 6 through line 11 in the upper left column on page 7)

(B) According to the matters described in (A) above, Exhibit Otsu 12 Document discloses that a promoter having a TAGACA sequence in the -35 region and a TATAAT sequence in the -10 region functioned in a coryneform bacterium, and enhanced the expression of the objective gene (the CAT gene) to 14 times compared to the level of a strain without the insertion of the promoter.

E. Regarding Exhibit Otsu 35 Document

(A) Matters described in Exhibit Otsu 35 Document

The contents described in Exhibit Otsu 35 Document include the following (For Tables 2, 10, 11, and 13, see Attachment 14 "Drawings of Cited Documents").

a. Prior art

"L-glutamic acid has a wide range of uses as a seasoning, and it is industrially produced by a fermentation method in which a glutamic acid-producing coryneform bacterium is cultured to cause it to produce L-glutamic acid, and the produced L-glutamic acid is separated from the culture of the bacterium." (Lines 10 through 14 in the lower left column on page 2)

"... attempts have been made to increase the production rate of L-glutamic acid and also efficiently produce L-glutamic acid by enhancing the activity of enzymes involved in glutamic acid biosynthesis, and the cloning of various enzyme genes involved in the biosynthetic pathway of glutamic acid is under way in order to achieve this objective." (Lines 13 through 19 in the lower right column on page 2)

b. Problem to be solved by the present invention

"However, no case has been reported so far regarding a strain capable of sufficiently improving the L-glutamic acid productivity in L-glutamic acid fermentation." (Lines 5 through 7 in the upper right column on page 3)

c. Means and actions for solving the problem

"As a result of intensive studies to solve the abovementioned problem, the present inventors succeeded in producing various strains wherein at least two or more types of enzyme genes, among the enzyme genes involved in the glutamic acid biosynthetic

pathway, were simultaneously enhanced by using recombinant DNA technology. When they performed glutamic acid fermentation by using these strains, they found that, in the case of performing glutamic acid fermentation by using a strain transformed with a recombinant DNA containing at least two or more types of enzyme genes derived from a glutamic acid-producing coryneform bacterium involved in the glutamic acid biosynthetic pathway, at least including the glutamate dehydrogenase (... GDH) gene and the isocitrate dehydrogenase (... ICDH) gene, not only the level of L-glutamic acid accumulated in the medium, but also the yield based on sugar improved remarkably compared to the case of using the parent strain, leading to the completion of the present invention." (Line 9 in the upper right column through line 6 in the lower left column on page 3)

"Recombinant plasmids to be used as materials for producing a multi-enhanced strain include pAG1001 containing the GDH gene, pAG3001 containing the ICDH gene, pA5001 containing the AH gene, and pAG4003 containing the CS gene. ... they are in the form of the vector plasmid pAG50 of a glutamic acid-producing coryneform bacterium into which a GDH gene-containing fragment, ICDH gene-containing fragment, AH gene-containing fragment, or CS gene-containing fragment is incorporated." (Lines 8 through 18 in the lower right column on page 6)

"Examples of the recombinant DNA carried by the glutamic acid-producing coryneform bacterium of the present invention include plasmids pIG101, pAIG321, pCIG231, pCAIG4 and the like mentioned below. pIG101 is a plasmid wherein the GDH gene and the ICDH gene derived from a glutamic acid-producing coryneform bacterium are simultaneously incorporated into pAG50. pAIG321 is a plasmid wherein the AH gene, the ICDH gene, and the GDH gene are simultaneously incorporated into pAG50. Also, pCIG231 is a plasmid wherein three enzymes, CS, ICDH, and GDH, are simultaneously incorporated into pAG50. Furthermore, pCAIG4 is a plasmid wherein four enzymes, CS, AH, ICDH, and GDH, have been simultaneously incorporated into pAG50." (Lines 7 through 19 in the upper left column on page 7)

d. Working Example 1

"This working example shows an example of producing a double-enhanced strain in which the activities of GDH and ICDH are simultaneously enhanced by using a glutamic acid-producing coryneform bacterium.

As recombinant plasmids containing the GDH gene and the ICDH gene, pAG1001 and pAG3001 were used respectively." (Lines 3 through 8 in the upper right column on page 8)

"*Corynebacterium melassecola* (...) carrying plasmid pAG1001 (⋯) was cultured in ...

The GDH activity was obtained by measuring ... The results are shown in Table 2." (Lines 19 through 18 in the upper left column on page 16)

e. Working Example 3

"The working example shows an example of producing a triple-enhanced strain in which CS + ICDH + GDH are enhanced. It also shows an example of producing a double-enhanced strain in which CS + ICDH are enhanced and a double-enhanced strain in which CS + GDH are enhanced.

As materials for producing a recombinant plasmid, the abovementioned pAG1001 and pAG3001 as well as recombinant plasmid pAG4003 containing the CS gene were used." (Lines 1 through 8 in the lower left column on page 35)

"(6) Measurement of the CS activity of plasmid pAG4001-carrying strain

Corynebacterium melassecola (...) 801 carrying plasmid pAG4001 was ... cultured. ... The CS activity was measured ... As a result, as shown in Table 10, the plasmid pAG4001-carrying strain showed higher CS specific activity than the vector pAG50-carrying strain and the strain carrying no plasmid." (Line 7 in the upper left column through line 4 in the upper right column on page 41)

"pCI31 and pCG5 are a recombinant plasmid simultaneously containing CS and ICDH and a recombinant plasmid simultaneously containing CS and GDH, respectively. The recombinant plasmid of CS + ICDH + GDH can be obtained by integrating a GDH fragment at the EcoRI breakpoint of pCI31, and more detailed analysis was performed regarding plasmid pCIG231 in the present invention." (Lines 12 through 18 in the lower right column on page 42)

"Cell extracts were prepared from host strains respectively carrying the pCI31, pCG5, and pCIG231 plasmids (*Corynebacterium melassecola* 801), and the enzyme activities of CS, ICDH, and GDH were compared with the case of using a cell extract of a host bacterium that does not carry any plasmids. ...

Based on the results in Table 11, it was confirmed that all target enzyme activities were enhanced in the pCI31-carrying strain, the pCG5-carrying strain, and the pCIG231-carrying strain." (Line 9 in the upper left column through line 15 in the upper right column on page 43)

f. Working Example 5

"This working example shows an example of glutamic acid fermentation using the multiple-enhanced strains obtained in Working Examples 1 through 4...

... The results obtained are shown in Table 13. ...

As is clear from Table 13, at least the strains in which both GDH and ICDH enzymes were simultaneously enhanced showed higher results in the amount of glutamic acid

accumulated and the yield based on sugar compared to those of the other strains." (Line 17 in the upper left column on page 44 through line 5 in the upper left column on page 45)

(B) According to the matters described in (A) above, Exhibit Otsu 35 Document discloses glutamic acid-producing coryneform bacteria in which, among the enzyme genes involved in the glutamic acid biosynthetic pathway, the GDH gene, the CS gene, the ICDH gene, etc. are enhanced by introducing plasmids carrying these genes, and discloses that, among these, glutamic acid-producing coryneform bacteria in which at least the GDH gene and the ICDH gene were simultaneously enhanced showed improvement in glutamic acid-producing ability. On the other hand, Table 13 of the document discloses that the glutamic acid productivity of strains in which the GDH gene or the CS gene was individually enhanced (9.2 g/dl) did not substantially differ from the productivity of their parent strain (9.1 g/dl).

(6) Whether or not Corrected Invention 1 lacks an inventive step based on Exhibit Otsu 6 Invention as the primary cited document

A. Regarding involvement of an inventive step in Corrected Invention 1-1

(A) Comparison between Exhibit Otsu 6 Invention and Corrected Invention 1-1

The following are found as a result of comparing Exhibit Otsu 6 Invention referred to in (3) B. above with Corrected Invention 1-1.

a. Common feature

They are inventions relating to a coryneform bacterium having specific nucleotide sequences in the -35 region and the -10 region of the promoter sequence of the GDH gene and/or having a specific nucleotide sequence in the -10 region of the promoter sequence of the CS gene on the chromosome of the coryneform bacterium.

b. Differences

Difference 1: The coryneform bacterium in Corrected Invention 1-1 has a TTGTCA sequence introduced into the -35 region and a TATAAT sequence introduced into the -10 region of the promoter sequence of the GDH gene (1-A'-1), whereas the coryneform bacterium in Exhibit Otsu 6 Invention has a TGGTCA sequence in the -35 region and a CATAAT sequence in the -10 region of the promoter sequence of the GDH gene.

Difference 2: The coryneform bacterium in Corrected Invention 1-1 has a TATAAT sequence introduced into the -10 region of the promoter sequence of the CS gene (1-A'-2), whereas the coryneform bacterium in Exhibit Otsu 6 Invention has a TAGCGT sequence in the -10 region of the promoter sequence of the CS gene.

Difference 3: Corrected Invention 1-1 is a method of producing glutamic acid by the fermentation method comprising the steps of cultivating a coryneform bacterium,

allowing glutamic acid to be accumulated in a medium, and collecting it from the medium (1-A-3, 1-B'), whereas Exhibit Otsu 6 Invention is an invention of a coryneform bacterium.

Meanwhile, with regard to Constituent Features 1-A'-1 and 1-A'-2 from among the constituent features of Corrected Invention 1-1, it suffices for either one to be fulfilled. Consequently, it can be said that if the configuration relating to either Difference 1 or Difference 2 could have been easily conceived of by a person skilled in the art, Corrected Invention 1-1 includes a configuration which could have been easily conceived of based on Exhibit Otsu 6 Invention.

(B) Regarding the ease in conceiving of Difference 1 (to adopt a TTGTCA sequence in the -35 region and a TATAAT sequence in the -10 region of the GDH gene)

a. Regarding suggestions in Exhibit Otsu 6 Document

(a) As mentioned in (3) B. above, Figure 1 of Exhibit Otsu 6 Document discloses nucleic acid sequences of the promoters of various genes of wild-type *Corynebacterium glutamicum*, and in these sequences, it discloses the respective promoter sequences of the GDH gene and the CS gene of the wild-type strain relating to Exhibit Otsu 6 Invention (a "TGGTCA" sequence in the -35 region and a "CATAAT" sequence in the -10 region of the promoter sequence of the GDH gene, and a "TGGCTA" sequence in the -35 region and a "TAGCGT" sequence in the -10 region of the promoter sequence of the CS gene).

In addition, Exhibit Otsu 6 Document describes that in relation to consensus sequences of the promoters of *Corynebacterium glutamicum* genes, in the promoter sequences of *Corynebacterium glutamicum* genes, conserved sequences are ttGcca.a in the -35 region and ggTA.aaT in the -10 region, and their positions, etc. correspond to consensus sequences in the -35 region and the -10 region of promoters of other Gram-positive and Gram-negative bacteria ((3) C. (A) above).

On the other hand, Exhibit Otsu 6 Document has no description suggesting that expression of the GDH gene and the CS gene, which are glutamic acid biosynthetic genes and specific genes on the chromosome of a coryneform bacterium, should be enhanced in order to improve the glutamic acid-producing ability in a method of producing glutamic acid by a fermentation method using a coryneform bacterium, or that, as a specific method therefor, the -35 region and the -10 region of the promoter sequences of these genes should be modified to make them closer to the consensus sequences of coryneform bacteria.

Consequently, as there is no motivation for a person skilled in the art to change the -35 region of the promoter of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention to a TTGTCA sequence and its -10 region to a

TATAAT sequence based on suggestions in Exhibit Otsu 6 Document, it is not found that a person skilled in the art could have easily conceived of the configuration relating to Difference 1.

(b) Regarding the Defendant's allegation

The Defendant alleges that matters including the following were known as common general technical knowledge as of Priority Date 1: [i] enhancement of glutamic acid biosynthetic genes, particularly enhancement of the GDH activity or the CS activity, was advantageous for improving the L-glutamic acid productivity of coryneform bacteria; and [ii] in *E. coli* and *Corynebacterium glutamicum*, expression of the objective gene was promoted by making the sequences in the -35 region and the -10 region of the promoter of the gene closer to the consensus sequences. On this basis, the Defendant alleges that it would have been easy for a person skilled in the art who read Exhibit Otsu 6 Document to conceive of changing the sequences of the -35 region and the -10 region of the promoter of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention to the consensus sequences of *Corynebacterium glutamicum* described in Exhibit Otsu 6 Document (a "TTGCCA" sequence in the -35 region and a "TAT.AT" sequence in the -10 region) or to sequences close to them in order to improve the glutamic acid-producing ability, and hence it would have been easy to conceive of adopting a TTGTCA sequence in the -35 region and a TATAAT sequence in the -10 region of the GDH gene.

However, even if the general actions of GDH and CS as enzymes involved in glutamic acid biosynthesis referred to in (8) D. (B) below, and, as described in Exhibit Otsu 9 Document ((4) B. (A) above), the fact that it is desirable to enhance the expression of genes such as the GDH gene and the CS gene involved in glutamic acid biosynthesis in order to improve the glutamic acid-producing ability of coryneform bacteria were known as of Priority Date 1, there is no sufficient evidence to find that a method to introduce specific mutations into specific regions of the promoters of these genes on the chromosome was known as a specific method for such enhancement.

In addition, even if it was known as of Priority Date 1 that there were cases where, in *E. coli*, expression of the objective gene was promoted by making the sequences in the -35 region and the -10 region of the promoter of the gene closer to the consensus sequences of the gene, as described in Exhibit Otsu 10 Document ((5) B. (B) above), there is no sufficient evidence to find that similar knowledge existed with regard to coryneform bacteria. In this regard, Exhibit Otsu 6 Document describes that there are cases where promoters of *E. coli* genes functioned in coryneform bacteria and suggests similarity between promoter regions of *Corynebacterium glutamicum* genes and promoter regions

of *E. coli* genes ((3) C. (B) above), but it also describes that there were multiple reports on coryneform bacteria-specific promoters which were obviously not functional in *E. coli* ((3) C. (B) above) and that the conservation levels of the abovementioned consensus motifs of *Corynebacterium glutamicum* were lower than those of *E. coli* ((3) C. (C) above), indicating that there are differences in the characteristics of the sequences in the promoter regions of genes between *Corynebacterium glutamicum* and *E. coli*. Moreover, as mentioned in (3) C. (D) above, Exhibit Otsu 6 Document describes, with regard to the relation between the activity of gene promoters and the process of making the sequences in their -35 region and -10 region similar to the consensus sequences, that the activity of promoters of *E. coli* genes can be correlated to a major extent with their similarity to the consensus sequences, but that no correlation could be recognized between the activity of promoters of *Corynebacterium glutamicum* genes and their similarity to the consensus sequences. Therefore, it cannot be said that the statements in Exhibit Otsu 6 Document concerning consensus sequences of *Corynebacterium glutamicum* and those concerning *E. coli* promoters, etc. suggest that, in order to enhance the objective gene of the coryneform bacterium, nucleotide sequences in the -35 region and the -10 region of the promoters of genes on the chromosome of the coryneform bacterium should be modified to the consensus sequences of coryneform bacteria or to sequences close to them.

Meanwhile, as mentioned in (3) A. (C) a. above, Exhibit Otsu 6 Document indicates the *tac* promoter and the *lac*UV5 promoter as *E. coli* promoters functional in coryneform bacteria, and as mentioned in (5) C. above, according to statements in Exhibit Otsu 11 Document, the sequence in the -10 region of these promoters is found to be a "TATAAT" sequence, while according to the statements in Exhibit Otsu 10 Document referred to in (5) B. above, this sequence is found to be the consensus sequence of *E. coli*. However, Exhibit Otsu 6 Document does not mention the impact of the *tac* promoter, etc. on the activity of the GDH gene or the CS gene of coryneform bacteria, and there is no sufficient evidence either to find the existence of common general technical knowledge that the GDH gene or the CS gene of coryneform bacteria could be enhanced by using these promoters (statements in Exhibit Otsu 64 Document, which the Defendant points out to be in relation to the functions of these promoters in coryneform bacteria, are statements relating to the activity of the CAT gene). By also taking into consideration the abovementioned statement in Exhibit Otsu 6 Document indicating that there are differences in the characteristics of the consensus sequences between *E. coli* and *Corynebacterium glutamicum*, it cannot be said that Exhibit Otsu 6 Document suggested introduction of a "TATAAT" sequence in the -10 region of the promoter of the GDH gene or the CS gene according to the sequence in the -10 region of the *tac* promoter, etc. or the

consensus sequence of *E. coli*.

Consequently, the Defendant's abovementioned allegation cannot be adopted.

b. Regarding combination with matters described in publicly known documents other than Exhibit Otsu 6 Document

While taking into account the suggestions in Exhibit Otsu 6 Document examined in a. above, an examination will be made as to whether it was easy for a person skilled in the art to conceive of the configuration relating to Difference 1 by combining matters described in other publicly known documents.

(a) Regarding matters described in Exhibit Otsu 9 Document

As mentioned in (4) B. (A) above, Exhibit Otsu 9 Document provides a description that the enhancement of glutamic acid biosynthetic genes is advantageous for improving the L-glutamic acid productivity, and provides an example in which the GDH gene and the CS gene are enhanced as an example of enhancement of glutamic acid biosynthetic genes.

On the other hand, the only specific disclosure of a method to enhance objective genes in Exhibit Otsu 9 Document is a method to ligate the objective genes at a position downstream from a strong promoter and introduce them as vectors, and the document does not disclose an example to add mutations into the promoter sequences of the objective genes on the chromosome ((4) B. (A), (B) above).

In this respect, with regard to introduction of a mutation into the promoter sequence of a gene on a chromosome, Exhibit Otsu 9 Document describes a method to introduce a mutation into the promoter sequence on a chromosome by utilizing homologous recombination as a method to obtain a coryneform bacterium deficient in α-KGDH activity ((4) A. (B) c. (c) above). However, it is merely described as a means for causing defects in α-KGDH activity, and not as a means for enhancing the glutamic acid biosynthetic enzyme activity of the GDH gene, etc. on the chromosome of a coryneform bacterium.

Apart from this, Exhibit Otsu 9 Document has statements on strong promoters that function in coryneform bacteria ((4) A. (B) c. (d) above), but it has no statements concerning the relation between sequences of these promoters and the consensus sequences of the promoters of genes of coryneform bacteria.

Consequently, it should be said that even if a person skilled in the art who came across Exhibit Otsu 6 Invention recognized the contents described in Exhibit Otsu 9 Document, motivation to introduce the sequence mentioned in Corrected Invention 1-1 into the promoter region of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention cannot be found.

Meanwhile, although the strong promoters that function in coryneform bacteria described in Exhibit Otsu 9 Invention include those of which -10 region is a "TATAAT" sequence (of those mentioned in (4) A. (B) c. (d) above, the sequence of the *tac* promoter described in Exhibit Otsu 11 Document referred to in (5) C. above), this point does not overturn the abovementioned conclusion, considering that Exhibit Otsu 9 Document does not describe introduction of a mutation into the promoter sequence of the objective gene on the chromosome as a method to enhance the objective gene as mentioned above.

(b) Regarding matters described in Exhibit Otsu 10 Document

Matters disclosed in Exhibit Otsu 10 Document are as mentioned in (5) B. (B) above. It discloses that, in *E. coli*, the promoter strength was enhanced in a mutant in which the sequence in the -35 region or the -10 region of the promoter was mutated to the consensus sequence and that the TTG sequence was conserved in the -35 region, but it makes no disclosure concerning sequences of the promoters of coryneform bacteria.

According to the results of the examination made in a. (b) above, even if a person skilled in the art who read Exhibit Otsu 6 Document recognized the contents described in Exhibit Otsu 10 Document concerning the promoter region of *E. coli*, motivation to introduce the sequence mentioned in Corrected Invention 1-1 into the promoter region of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention in order to enhance the objective gene of the coryneform bacterium cannot be found.

(c) Regarding matters described in Exhibit Otsu 11 Document

Matters disclosed in Exhibit Otsu 11 Document are as mentioned in (5) C. above. It discloses that, in *E. coli*, the -35 region and the -10 region of the *tac* promoter sequence are TTGACA and TATAAT, respectively, and that the -35 region and the -10 region of the *lac*UV5 promoter sequence are TTTACA and TATAAT, respectively, but the disclosure is neither related to the sequences of the promoters of coryneform bacteria nor related to the consensus sequences of the promoters of genes of coryneform bacteria.

As mentioned in (3) A. (C) a. above, even if the mention of the *tac* promoter and the *lac*UV5promoter in Exhibit Otsu 6 Document is taken into consideration, according to the results of the examination made in a. (b) above, motivation cannot be found for a person skilled in the art to introduce the sequence mentioned in Corrected Invention 1-1 into the promoter region of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention in order to enhance the objective gene of the coryneform bacterium based on the contents described in Exhibit Otsu 6 Document and Exhibit Otsu 11 Document.

(d) Regarding matters described in Exhibit Otsu 12 Document

Matters disclosed in Exhibit Otsu 12 Document are as mentioned in (5) D. (B) above. It discloses that a promoter having a TAGACA sequence in the -35 region and a TATAAT sequence in the -10 region functioned in a coryneform bacterium, and enhanced the expression of the objective gene (the CAT gene) to 14 times compared to the level of a strain without the insertion of the promoter.

On the other hand, Exhibit Otsu 12 Document merely describes the results of the examination on the relation between a promoter having a specific sequence and the activity of a specific gene (the CAT gene), and it neither discloses nor suggests the consensus sequences in the -35 region and the -10 region of the promoters of coryneform bacteria and also does not disclose a strain in which a mutation has been introduced into the promoter sequence of a gene on the chromosome of a coryneform bacterium.

Consequently, even if a person skilled in the art who read Exhibit Otsu 6 Document recognized the contents described in Exhibit Otsu 12 Document, motivation to introduce the sequence mentioned in Corrected Invention 1-1 into the promoter region of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention in order to enhance the objective gene of the coryneform bacterium cannot be found.

(e) Regarding matters described in Exhibit Otsu 35 Document

According to (5) E. (A) above, Exhibit Otsu 35 Document discloses an example in which glutamic acid biosynthetic genes, including the GDH gene, the CS gene, and the ICDH gene, are enhanced as a method to improve the glutamic acid productivity.

On the other hand, in Exhibit Otsu 35 Document, the method specifically disclosed as a method to enhance these objective genes is a method of introducing plasmids (vectors) carrying these genes, and there is no statement about adding mutations to the promoter sequences of genes on the chromosome ((5) E. (A), (B) above).

Moreover, Exhibit Otsu 35 Document also discloses that the glutamic acid-producing ability improved in strains in which at least the GDH gene and the ICDH gene have been simultaneously enhanced, and that the glutamic acid-producing ability of strains in which the GDH gene or the CS gene was individually enhanced did not substantially differ from that of their parent strain. Hence, it cannot be said that Exhibit Otsu 35 Document suggests a method of individually or simultaneously enhancing the GDH gene and the CS gene as a method to improve the glutamic acid-producing ability.

Consequently, even if a person skilled in the art who read Exhibit Otsu 6 Document recognized the contents described in Exhibit Otsu 35 Document, motivation to introduce the sequence mentioned in Corrected Invention 1-1 into the promoter region of the GDH gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention in order to enhance the objective gene of the coryneform bacterium cannot be found.

c. According to the results of the examination made in a. and b. above, a person skilled in the art is not found to have been able to easily conceive of the configuration relating to Difference 1 based on Exhibit Otsu 6 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in b. above and other common general technical knowledge.

(C) Regarding the ease in conceiving of Difference 2 (to adopt a TATAAT sequence in the -10 region of the CS gene)

Similarly to (B) a. above, there is no motivation for a person skilled in the art to change the -10 region of the promoter of the CS gene on the chromosome of *Corynebacterium glutamicum* in Exhibit Otsu 6 Invention to a TATAAT sequence based on suggestions in Exhibit Otsu 6 Document. In addition, according to the contents described in the respective documents referred to in (B) b. above, even if a person skilled in the art who read Exhibit Otsu 6 Document recognized these contents, there is also no motivation for the person to introduce the abovementioned mutation into the coryneform bacterium in Exhibit Otsu 6 Invention.

Meanwhile, as mentioned in (5) A. (B) above, Exhibit Otsu 8 Document discloses that the sequence in the -10 region of the promoter of the CS gene of wild-type *Corynebacterium glutamicum* was deduced as being TATAGC, unlike in Exhibit Otsu 6 Invention. However, even if a person skilled in the art who read Exhibit Otsu 6 Document recognized this statement, there is no motivation for the person to change the sequence in the -10 region of the promoter of the CS gene on the chromosome of the coryneform bacterium in Exhibit Otsu 6 Invention to a TATAAT sequence, similar to the above.

Consequently, a person skilled in the art is not found to have been able to easily conceive of the configuration relating to Difference 2 based on Exhibit Otsu 6 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in (5) above and other common general technical knowledge.

(D) As mentioned in (B) and (C) above, neither the configuration relating to Difference 1 nor that relating to Difference 2 could have been easily conceived of based on Exhibit Otsu 6 Invention. Consequently, without having to make determinations on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-1 based on Exhibit Otsu 6 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 6 Invention as the primary cited document is not found for Corrected Invention 1-1.

B. Regarding involvement of an inventive step in Corrected Invention 1-2

The configuration of Corrected Invention 1-2 is included in the configuration of Corrected Invention 1-1, and when Exhibit Otsu 6 Invention referred to in (3) B. above and Corrected Invention 1-2 are compared, they differ in respect to Difference 1 and Difference 3 referred to in A. (A) b. above.

In addition, similarly to A. (B) above, a person skilled in the art is not found to have been able to easily conceive of the configuration relating to Difference 1 based on Exhibit Otsu 6 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in A. (B) b. above and other common general technical knowledge.

Consequently, without having to make determinations on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-2 based on Exhibit Otsu 6 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 6 Invention as the primary cited document is not found for Corrected Invention 1-2.

C. Regarding involvement of an inventive step in Corrected Invention 1-3

The configuration of Corrected Invention 1-3 is included in the configuration of Corrected Invention 1-1, and when Exhibit Otsu 6 Invention referred to in (3) B. above and Corrected Invention 1-2 are compared, they differ at least in respect to Difference 1, Difference 2, and Difference 3 referred to in A. (A) b. above.

In addition, similarly to A. (B) and (C) above, a person skilled in the art is not found to have been able to easily conceive of either the configuration relating to Difference 1 or that relating to Difference 2 based on Exhibit Otsu 6 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in A. (B) b. and A. (C) above and other common general technical knowledge.

Consequently, without having to make determinations on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-3 based on Exhibit Otsu 6 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 6 Invention as the primary cited document is not found for Corrected Invention 1-3.

(7) Whether or not Corrected Invention 1 lacks an inventive step based on Exhibit Otsu 9 Invention as the primary cited document

A. Regarding involvement of an inventive step in Corrected Invention 1-1

(A) Comparison between Exhibit Otsu 9 Invention and Corrected Invention 1-1

The following are found as a result of comparing Exhibit Otsu 9 Invention referred to in (4) B. (B) above with Corrected Invention 1-1.

a. Common feature

They are methods of producing L-glutamic acid by the fermentation method comprising the steps of cultivating, in a medium, a coryneform bacterium in which a mutation has been introduced into the nucleotide sequence of the promoter of a gene on the chromosome, to allow L-glutamic acid to be produced and accumulated in the medium, and collecting it from the medium.

b. Differences

Difference 1: The coryneform bacterium in Corrected Invention 1-1 has a TTGTCA sequence introduced into the -35 region and a TATAAT sequence introduced into the -10 region of the promoter sequence of the GDH gene on the chromosome (1-A'-1), whereas in the coryneform bacterium in Exhibit Otsu 9 Invention, no such specification is made, and a vector wherein the GDH gene is ligated at a position downstream from a strong promoter has been introduced into the bacterium.

Difference 2: The coryneform bacterium in Corrected Invention 1-1 has a TATAAT sequence introduced into the -10 region of the promoter sequence of the CS gene on the chromosome (1-A'-2), whereas in the coryneform bacterium in Exhibit Otsu 9 Invention, no such specification is made, and a vector wherein the CS gene is ligated at a position downstream from a strong promoter has been introduced into the bacterium.

Meanwhile, with regard to Constituent Features 1-A'-1 and 1-A'-2 from among the constituent features of Corrected Invention 1-1, it suffices for either one to be fulfilled. Consequently, it can be said that if the configuration relating to either Difference 1 or Difference 2 could have been easily conceived of, Corrected Invention 1-1 includes a configuration which could have been easily conceived of by a person skilled in the art based on Exhibit Otsu 9 Invention.

(B) Regarding the ease in conceiving of Difference 1 (to introduce a TTGTCA sequence in the -35 region and a TATAAT sequence in the -10 region of the promoter sequence of the GDH gene on the chromosome)

a. According to the results of the examination made in (6) A. (B) b. (a) above, Exhibit Otsu 9 Document provides a description that enhancement of glutamic acid biosynthetic genes is advantageous for improving the L-glutamic acid productivity, and provides an example in which the GDH gene and the CS gene are enhanced as an example of enhancement of glutamic acid biosynthetic genes. However, the only specific disclosure of a method to enhance the GDH gene and the CS gene, etc. in Exhibit Otsu 9 Document is a method to ligate these genes at a position downstream from a strong promoter and

introduce them as vectors, and the document does not disclose an example to add mutations into the promoter sequences of the objective genes on the chromosome. The document contains no statements that would motivate modification of the nucleotide sequences in the -35 region and the -10 region of the promoter sequences of the GDH gene and the CS gene, which are glutamic acid biosynthetic genes and specific genes on the chromosome of a coryneform bacterium, to make them closer to the consensus sequences of coryneform bacteria.

Consequently, even if a person skilled in the art who read Exhibit Otsu 9 Invention recognized the contents described in Exhibit Otsu 6 Document, Exhibit Otsu 10 Document, Exhibit Otsu 11 Document, Exhibit Otsu 12 Document, or Exhibit Otsu 35 Document examined in (3), (5), and (6) A. (B) above, there is no motivation in Exhibit Otsu 9 Invention for a person skilled in the art to pay attention to the sequences in the -35 region and the -10 region of the promoter sequence of the GDH gene and the extent of enhancement of the expression level of the objective gene as well as their relevance to improving the glutamic acid-producing ability, or to introduce the sequences mentioned in Corrected Invention 1-1 into the -35 region and the -10 region of the promoter sequence of the GDH gene on the chromosome of the coryneform bacterium in order to obtain a mutant strain having the ability to produce glutamic acid in high yield, and it is not found that a person skilled in the art could have easily conceived of the configuration relating to Difference 1.

b. The Defendant alleges that the process of enhancing the expression of the GDH gene or the CS gene, not on a vector, but on a chromosome by using a promoter having a specific sequence in order to solve problems that occur due to the use of a vector is merely a matter that could have been easily conceived of by a person skilled in the art. However, as common general technical knowledge as of Priority Date 1, it was known that the method of enhancing the objective gene by using a vector involved the problems described in paragraphs [0002] and [0003] of Description 1, including the facts that there are often cases in which the expression level of the objective gene becomes too high, leading to extremely restrained growth or a decline in the target substance-producing ability, and that loss of the plasmid itself occurs. Therefore, even if a method to introduce a mutated gene on a chromosome by utilizing homologous recombination was known (however, as mentioned in (4) A. (B) c. (c) above, Exhibit Otsu 9 Document described it as a means for causing defects in α-KGDH activity, and not as a means for enhancing a gene), in light of the abovementioned contents described in Exhibit Otsu 9 Document and the publicly known documents indicated by the Defendant, it should be said that there is no motivation for a person skilled in the art who came across Exhibit Otsu 9 Invention to

modify the sequences at specific positions of the promoters of the GDH gene and the CS gene of a coryneform bacterium to make them closer to the consensus sequences of coryneform bacteria, and hence this does not overturn the conclusion mentioned in a. above.

(C) Regarding the ease in conceiving of Difference 2 (to introduce a TATAAT sequence in the -10 region of the promoter sequence of the CS gene on the chromosome)

Due to the same reason as in (B) above, even if a person skilled in the art who came across Exhibit Otsu 9 Invention recognized the contents described in Exhibit Otsu 6 Document, Exhibit Otsu 8 Document, Exhibit Otsu 10 Document, Exhibit Otsu 11 Document, Exhibit Otsu 12 Document, or Exhibit Otsu 35 Document examined in (3), (5), and (6) A. (B) and (C) above, there is no motivation in Exhibit Otsu 9 Invention for a person skilled in the art to pay attention to the sequence in the -10 region of the promoter of the CS gene and the extent of enhancement of the expression level of the objective gene as well as their relevance to improving the glutamic acid-producing ability, or to introduce the sequence mentioned in Corrected Invention 1-1 into the -10 region of the promoter of the CS gene on the chromosome in order to obtain a mutant strain having the ability to produce glutamic acid in high yield, and it is not found that a person skilled in the art could have easily conceived of the configuration relating to Difference 2.

(D) As mentioned in (B) and (C) above, neither the configuration relating to Difference 1 nor that relating to Difference 2 could have been easily conceived of based on Exhibit Otsu 9 Invention. Consequently, without having to make determinations on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-1 based on Exhibit Otsu 9 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 9 Invention as the primary cited document is not found for Corrected Invention 1-1.

B. Regarding involvement of an inventive step in Corrected Invention 1-2

The configuration of Corrected Invention 1-2 is included in the configuration of Corrected Invention 1-1, and when Exhibit Otsu 9 Invention referred to in (4) B. (B) above and Corrected Invention 1-2 are compared, they differ at least in respect to Difference 1 referred to in A. (A) b. above.

In addition, similarly to A. (B) above, a person skilled in the art is not found to have been able to easily conceive of the configuration relating to Difference 1 based on Exhibit Otsu 9 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in (3), (5), and (6) A. (B) above and other common general technical knowledge.

Consequently, without having to make determination on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-2 based on Exhibit Otsu 9 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 9 Invention as the primary cited document is not found for Corrected Invention 1-2.

C. Regarding involvement of an inventive step in Corrected Invention 1-3

The configuration of Corrected Invention 1-3 is included in the configuration of Corrected Invention 1-1, and when Exhibit Otsu 9 Invention referred to in (4) B. (B) above and Corrected Invention 1-3 are compared, they differ at least in respect to Difference 1 and Difference 2 referred to in A. (A) b. above.

In addition, similarly to A. (B) and (C) above, a person skilled in the art is not found to have been able to easily conceive of either the configuration relating to Difference 1 or that relating to Difference 2 based on Exhibit Otsu 9 Document as of Priority Date 1, by combining the contents of the document with the contents described in the publicly known documents referred to in (3), (5), and (6) A. (B) and (C) above and other common general technical knowledge.

Consequently, without having to make determinations on other points, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 1-3 based on Exhibit Otsu 9 Invention as the primary cited document, and hence a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 9 Invention as the primary cited document is not found for Corrected Invention 1-3.

(8) Whether or not Corrected Invention 1 violates the enablement requirement or the support requirement

A. Problem to be solved by Corrected Invention 1

The problem to be solved by Corrected Invention 1 is, as mentioned in (1) B. (B) above, to provide a process for constructing a mutant strain capable of appropriately enhancing and regulating the expression level of the objective gene and that has the ability to produce amino acids in high yield by genetic recombination or mutation, without using a plasmid, and to provide a glutamic acid fermentation method for increasing the yield of glutamic acid and producing glutamic acid at a lower cost, using a coryneform glutamic acid-producing bacterium (paragraph [0006]).

B. Regarding the process for producing L-arginine

As Corrected Inventions 1-1, 1-2, and 1-3 all no longer include the process for producing L-arginine due to Correction 1, the violation of the support requirement

regarding the process for producing L-arginine mentioned in 5. (2) above is found to have been eliminated by Correction 1 with regard to these inventions.

Similarly, Corrected Invention 1-1, 1-2, and 1-3 are not found to violate the enablement requirement in relation to the process for producing L-arginine either.

C. Regarding introduction of a mutation into the promoter of the ICDH gene, the PDH gene, or the argininosuccinate synthase gene

Although Invention 1-1 before correction included inventions using a coryneform bacterium in which a specific nucleotide sequence has been introduced into the ICDH gene, the PDH gene, or the argininosuccinate synthase gene, Corrected Invention 1-1 no longer includes these inventions due to Correction 1.

Consequently, Corrected Invention 1-1 is not found to violate either the support requirement or the enablement requirement in relation to introduction of a mutation into the promoter of the ICDH gene, the PDH gene, or the argininosuccinate synthase gene.

D. Regarding introduction of a mutation into the promoter of the GDH gene

(A) Statements in Description 1

As mentioned in (1) B. above, the detailed explanation of the invention in Description 1 states the following: [i] in order to solve the problem mentioned in A. above, a configuration can be adopted in which a mutant of a coryneform bacterium is prepared by causing or introducing by genetic recombination a mutation to the promoter sequence of an amino acid biosynthetic gene on the chromosome of the coryneform bacterium to make the sequence closer to the consensus sequence, then that mutant is cultured, and as a result, only a mutant that produces a large amount of the target amino acid is collected (paragraph [0007]), while for examples of enzymes involved in the biosynthesis of amino acid, enzymes such as GDH and CS are effective in the case of glutamic acid fermentation (paragraph [0012]), and the abovementioned mutation may be caused either only for the promoter sequence of one gene or for the promoter sequences of two or more genes (paragraph [0015]); and [ii] as an example of configuration of such mutation, it is preferable that, in the promoter sequence of the GDH gene, the -35 region is TTGTCA or the -10 region is TATAAT (paragraph [0017]).

As mentioned in (1) B. above, Description 1 discloses the following: [iii] Working Example 2 describes a method to produce a strain wherein the -35 region of the promoter sequence of a GDH gene of the parent strain (AJ13029) has been mutated to a TTGTCA sequence, and the -10 region of the promoter sequence has been mutated to a TATAAT sequence, as "FGR2," and the fact that the specific activity of GDH of FGR2 is about 3.4 times that of the parent strain, while its L-glutamic acid-producing ability also increased compared to the parent strain (paragraphs [0026], [0029], [0031], and [0032], [Table 5],

[Table 6]); and [iv]Working Example 7 also describes a method to produce a strain wherein the same mutation as that in "FGR2" has been introduced into the GDH gene on the chromosome ("GA02"), and the fact that the specific activity of GDH of GA02 is about 3.5 times that of the parent strain, while its L-glutamic acid-producing ability increased compared to the parent strain (paragraphs [0087] through [0089], [Table 23]).

(B) Common general technical knowledge concerning actions of GDH and CS in the glutamic acid production process

According to Exhibit Otsu 17 (*Iwanami Seibutsugaku Jiten* [Iwanami biology dictionary], 4th ed., published on July 12, 1996) and the entire import of oral arguments, the following common general technical knowledge is found to have existed as of the time of the filing of the application for Patent Right 1 with regard to involvement of GDH and CS in glutamic acid biosynthesis.

a. The citric acid cycle (TCA cycle) is involved in glutamic acid biosynthesis by way of supplying materials for synthesis of glutamic acid.

b. The TCA cycle is an oxidation process which completely degrades acetyl CoA produced by glycolysis and other catabolic action into water and carbon dioxide. It consists of eight steps and repeats the cycle where acetyl CoA is condensed with oxaloacetic acid to form citric acid, which is then converted into isocitric acid, α-ketoglutaric acid, succinyl CoA, succinic acid, fumaric acid, L-malic acid, and oxaloacetic acid in order, and citric acid is produced again.

c. The citric acid-producing enzyme (CS) is an enzyme which condenses oxaloacetic acid with acetyl CoA, and catalyzes a reaction to synthesize citric acid in the TCA cycle.

d. L-glutamic acid is produced from α-ketoglutaric acid, which is an intermediate product in the TCA cycle. GDH is an enzyme which reductively fixes ammonia to α-ketoglutaric acid and catalyzes a reaction to produce L-glutamic acid.

(C) According to the statements in Description 1 mentioned in (A) above and the common general technical knowledge mentioned in (B) above, it is found that a person skilled in the art who read Description 1 could have recognized that the problem mentioned in A. above, which is to provide a process for constructing a mutant strain capable of appropriately enhancing and regulating the expression level of the objective gene and that has the ability to produce amino acids in high yield, without using a plasmid, could be solved by the method of producing L-glutamic acid wherein a mutation has been introduced into the promoter of the GDH gene in Corrected Invention 1, that is, a method to introduce a TTGTCA sequence in the -35 region and a TATAAT sequence in the -10 region of the promoter sequence of the GDH gene on the chromosome of a coryneform bacterium used for producing glutamic acid.

In addition, according to the statements in Description 1 mentioned above, it is found that, based on these statements, a person skilled in the art could have been able to obtain a strain in which the abovementioned sequences are respectively introduced into the -35 region and the -10 region of the promoter sequence of the GDH gene on the chromosome of a coryneform bacterium and work Corrected Invention 1 without undergoing excessive trial and error.

Consequently, Corrected Invention 1 is not found to violate either the support requirement or the enablement requirement in relation to the introduction of a mutation into the promoter of the GDH gene mentioned above.

E. Regarding introduction of a mutation into the promoter of the CS gene

(A) Statements in Description 1

As mentioned in (1) B. above and D. above, the detailed explanation of the invention in Description 1 states, in addition to the statements mentioned in [i] in D. above, the fact that examples of the configuration of a mutation that could be adopted for solving the problem mentioned in A. above include one that has the TTGACA sequence in the -35 region and/or the TATAAT sequence in the -10 region of the promoter sequence of the CS gene (paragraph [0018]). Then, after describing Working Example 2 relating to "FGR2" wherein the glutamic acid-producing ability has been improved by introducing mutations into the promoter sequence of the GDH gene mentioned in [iii] in D. above, it describes, in Working Example 3, a method of producing "GB02" wherein the promoter sequence of the GDH gene on the chromosome of the abovementioned "FGR2" in Working Example 2 remains the same, but a mutation has been introduced to make the -10 region of the promoter sequence of the CS gene a TATAAT sequence, and a method of producing "GB03" wherein mutations have been introduced to make the -35 region of the promoter sequence a TTGACA sequence and the -10 region of the promoter sequence a TATAAT sequence, as well as the fact that the specific activity of CS of GB02 is about 1.9 times that of FGR2 and the specific activity of CS of GB03 is about 4.0 times that of FGR2, and that their L-glutamic acid-producing ability both further improved compared to FGR2.

(B) Regarding Corrected Invention 1-1

a. Comprehensively considering the statements in Description 1 mentioned in (A) above and the common general technical knowledge mentioned in D. (B) above, it is found that a person skilled in the art who read Description 1 could have recognized that the problem mentioned in A. above, which is to provide a process for constructing, without using a plasmid, a mutant strain capable of appropriately enhancing and regulating the expression level of the objective gene and that has the ability to produce amino acids in high yield, could be solved by the method of producing L-glutamic acid wherein a mutation has been

introduced into the promoter of the CS gene in Corrected Invention 1-1, that is, a method to introduce a TATAAT sequence in the -10 region of the promoter sequence of the CS gene on the chromosome of a coryneform bacterium used for producing glutamic acid. In addition, according to the statements in Description 1 mentioned in (A) above, it is found that, based on these statements, a person skilled in the art could have been able to obtain a strain in which the abovementioned sequence is introduced into the -10 region of the promoter sequence of the CS gene on the chromosome of a coryneform bacterium and work Corrected Invention 1-1 without undergoing excessive trial and error.

b. Regarding the Defendant's allegations

(a) The Defendant alleges, with regard to a configuration included in Corrected Invention 1-1 which uses a strain wherein no mutation is introduced into the promoter sequence of the GDH gene and a mutation is only introduced into the promoter sequence of the CS gene (a configuration that fulfills Constituent Feature 1-A'-2, but not Constituent Feature 1-A'-1), that Description 1 contains no working example wherein a mutation is only introduced into the promoter sequence of the CS gene, and it was known in documents published before the filing of the application (Exhibit Otsu 8 Document, Exhibit Otsu 35 Document) that the glutamic acid productivity would not improve by enhancing the CS activity without enhancing the GDH activity in a coryneform bacterium, and hence a person skilled in the art could not have recognized that the glutamic acid productivity would improve based on the statements in Description 1 and the common general technical knowledge as of the time of the filing of the application.

(b) Indeed, as mentioned in (A) above, GB02 and GB03, wherein a mutation has been introduced into the promoter of the CS gene on the chromosome, in Working Example 3, were strains produced by introducing a further mutation into FGR2 in Working Example 2, and therefore, the mutation mentioned in Working Example 2 has been introduced not only into the promoter of the CS gene, but also into the promoter sequence of the GDH gene, which means that Description 1 contains no working example relating to a strain wherein a mutation is only introduced into the promoter sequence of the CS gene. However, as mentioned in (A) above, Working Example 3 shows the results of comparing FGR2 with GB02 and GB03, wherein the promoter sequence of the GDH gene of FGR2 remains the same as in Working Example 2 and a mutation is further introduced to make the -10 region of the promoter sequence of the CS gene a TATAAT sequence. Therefore, it can be said that Working Example 3 discloses the impact which the method of introducing the abovementioned sequence into the promoter sequence of the CS gene on the chromosome has on the glutamic acid-producing ability.

(c) Moreover, the Defendant alleges that it was known since before the filing of the

application that the glutamic acid productivity would not improve by enhancing the CS activity without enhancing the GDH activity in a coryneform bacterium.

While the contents described in Exhibit Otsu 8 Document indicated by the Defendant are as mentioned in (5) A. above, the document discloses that overexpression of the CS gene slows the growth of *Corynebacterium glutamicum* and that in *Corynebacterium glutamicum* in which CS activity was increased by introducing plasmids the glutamic acid productivity was not improved compared to a strain to which no mutation was added. In addition, Exhibit Otsu 35 Document discloses, as mentioned in (5) E. above, that with regard to glutamic acid-producing coryneform bacteria in which the GDH gene, the CS gene, the ICDH gene, etc. are enhanced by introducing plasmids carrying these genes, the glutamic acid-producing ability of strains in which the GDH gene or the CS gene was individually enhanced did not substantially differ from that of their parent strain.

However, in abovementioned Exhibit Otsu 8 Document and Exhibit Otsu 35 Document, the examples where the glutamic acid-producing ability did not improve by enhancing the CS gene individually were all examples in which the CS gene was enhanced by introducing plasmids, and the method of enhancement in these examples differs from that employed in Corrected Invention 1-1, which is to change the promoter sequence of the objective gene on the chromosome. Moreover, regarding enhancement of genes by introducing plasmids, Exhibit Otsu 35 Document shows a result that the glutamic acid-producing ability also did not improve compared to the parent strain in the case of enhancing the GDH gene individually, and this also differs from the experiment results of FGR2, where the GDH gene was individually enhanced by changing the sequence of the promoter of the objective gene on the chromosome, as shown in Working Example 2 in Description 1. According to statements in paragraphs [0002] and [0003] of Description 1 and the entire import of oral arguments, with regard to enhancement of the objective gene by plasmids, it is found to have been known since before the filing of the application that there are cases in which the expression level of the objective gene becomes too high, leading to extremely restrained growth or a decline in the target substance-producing ability. When this point is also taken into consideration, even when the same objective gene is enhanced in a coryneform bacterium, the impact on improvement of the glutamic acid-producing ability could differ depending on the enhancement method. Thus, it cannot be said, based on the statements in Exhibit Otsu 8 Document and Exhibit Otsu 35 Document, that the glutamic acid-producing ability does not improve by enhancing the CS gene individually, when the method of introducing plasmids is not adopted, and there is no other evidence sufficient to find that there was such common general technical knowledge as of the time of the filing of the application.

(d) According to (a) through (c) above, the conclusion mentioned in a. above cannot be overturned even by taking into consideration the Defendant's allegations.

(C) Regarding Corrected Invention 1-3

Similarly to (B) above, it is found that, based on the statements in Description 1 mentioned in (A) above and the common general technical knowledge mentioned in D. (B) above, a person skilled in the art who read Description 1 could have recognized that the problem mentioned in A. above, which is to provide a process for constructing a mutant strain capable of appropriately enhancing and regulating the expression level of the objective gene and that has the ability to produce amino acids in high yield, without using a plasmid, could be solved by the method of producing L-glutamic acid wherein a mutation has been introduced into the promoter of the CS gene in Corrected Invention 1-3, that is, a method to introduce a TATAAT sequence in the -10 region of the promoter sequence of the CS gene on the chromosome of a coryneform bacterium used for producing glutamic acid or a method to introduce a TTGACA sequence in the -35 region and a TATAAT sequence in the -10 region of the promoter sequence. In addition, according to the statements in Description 1 mentioned in (A) above, it is found that, based on these statements, a person skilled in the art could have been able to obtain a strain in which the abovementioned sequences are introduced into the promoter sequence of the CS gene on the chromosome of a coryneform bacterium and work Corrected Invention 1-3 without undergoing excessive trial and error.

F. Regarding the promoter sequence of *Corynebacterium glutamicum* wild-type strain ATCC13869

The Defendant indicates that the sequences in regions surrounding the -35 region and the -10 region of the promoter sequence of SEQ ID NO: 1 in paragraph [0025] of Description 1 in Working Example 1 differ from the sequences of the wild-type strain ATCC13869 described in Exhibit Ko 22 at two positions, and alleges that the experiment in Description 1 was performed based not on wild-type strain ATCC13869 but a different strain, and therefore it cannot be said that Description 1 has demonstrated a change in activity caused by mutating the promoter sequence of a wild-type strain, at least with regard to the GDH gene.

As mentioned in (1) B. (D) a. above, however, in Working Example 1, a plasmid wherein a mutation has been introduced into the promoter sequence of the GDH gene has been produced, but a mutation has not been introduced into the promoter sequence of the GDH gene on the chromosome of a coryneform bacterium, and as mentioned in D. (A) above, the improvement of the glutamic acid-producing ability in a strain wherein the sequences prescribed in Corrected Invention 1 have been introduced into the promoter

sequence of the GDH gene on the chromosome was confirmed in Working Example 2 and Working Example 7.

The nucleotide sequences of the promoter region of the GDH gene of the AJ13029 strain and of FGR1 and FGR2, which are AJ13029 strains into which mutations have been introduced, used in Working Example 2 are described in [Table 6] of Description 1, but the Defendant has not made any particular indication about these sequences, and similarly, the Defendant has not made any particular indication about the strain into which a mutation has been introduced in Working Example 7.

If so, it cannot be said that the Defendant's abovementioned indications about the statements on the promoter sequence in Working Example 1 affect the conclusion mentioned in D. above to the effect that Corrected Invention 1 fulfills the support requirement and the enablement requirement in relation to the introduction of a mutation into the promoter of the GDH gene.

With regard to the statements on the promoter sequence of ATCC13869 in Working Example 1, the Defendant has not made specific allegations regarding violation of the support requirement and the enablement requirement, apart from making the abovementioned allegations in relation to introduction of a mutation into the GDH gene. Consequently, Corrected Invention 1 is not found to violate the support requirement or the enablement requirement based on the abovementioned statements on the sequence.

G. Regarding regions surrounding the -35 region and the -10 region of the promoter

The Defendant alleges that, as Corrected Invention 1 does not specify the sequences in regions surrounding the -35 region and the -10 region of the GDH gene and the CS gene, a person skilled in the art cannot recognize that the glutamic acid productivity would improve by making the regions surrounding the -35 region and the -10 region arbitrary sequences or lengths, even by referring to the working examples and other statements in Description 1 as well as common general technical knowledge, and hence the support requirement is not fulfilled, and due to the same reason, the statements in the detailed explanation of the invention in Description 1 do not fulfill the enablement requirement.

As mentioned in (1) B. (D) b. above, "FGR2" in Working Example 2 is a strain wherein mutations are introduced to mutate the -35 region and the -10 region of the promoter sequence of the GDH gene of the parent strain, AJ13029, to the sequences mentioned in Corrected Invention 1. Meanwhile, [Table 6] of Description 1 shows that the sequence in the region between the -35 region and the -10 region of the promoter sequence of the GDH gene of "FGR2" is the same as that of the parent strain, AJ13029, except for deletion of one nucleotide, and apart from this, Description 1 contains no statements to the effect that a mutation has been added to the sequence of the parent strain

with regard to the sequences in the regions surrounding the -35 region and the -10 region of the promoter of GDH of FGR2.

In addition, also with regard to the "GB02" and "GB03" strains in Working Example 3 and the "GA02" strain in Working Example 7, as examined in D. and E. above, Description 1 contains no statements to the effect that a mutation has been added to the sequences in the regions surrounding the -35 region and the -10 region of the promoter of the CS gene or the GDH gene when producing the respective strains.

According to the above, it should be said that, even if the fact that the sequences and lengths of the regions surrounding the -35 region and the -10 region affect the promoter activity was publicly known, a person skilled in the art who read Description 1 could have understood that, in Corrected Invention 1 for which the problem to be solved is to introduce on the chromosome a mutation to make the -35 region or the -10 region of the promoter sequence of the GDH gene or the CS gene a specific sequence, the regions surrounding the -35 region and the -10 region of the promoter sequence, which are not specified in the invention, can be the same as those in a non-modified strain, as alleged by the Defendant.

Consequently, it cannot be said, based on the fact that the sequences in the regions surrounding the -35 region and the -10 region have not been specified, that Corrected Invention 1 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention and the common general technical knowledge as of the time of the filing of the application. Therefore, the Defendant's allegation of a violation of the support requirement relating to this point is groundless. Also, for the same reason, it can be said that a person skilled in the art can work Corrected Invention 1 without undergoing excessive trial and error based on the statements in Description 1, even if the sequences in the regions surrounding the -35 region and the -10 region of the promoter sequence have not been specified, and hence, the Defendant's allegation of a violation of the enablement requirement relating to this point is also groundless.

(9) Summary on the defense of invalidity concerning Patent Right 1

A. Regarding whether or not the requirements for re-defense of correction are fulfilled

While Correction 1 satisfies the requirements for correction as mentioned in No. 2, 2. (4) above, Defendant's Production Process 1 literally falls within the technical scopes of Corrected Invention 1-1 (corresponding to Invention 1-1), Corrected Invention 1-2 (corresponding to Invention 1-2), and Corrected Invention 1-3 (corresponding to Invention 1-4), whereas Defendant's Production Process 3 literally falls within the technical scope of Corrected Invention 1-1 but does not fall within the technical scope of

Corrected Invention 1-3, as mentioned in (2) above.

Among the grounds for invalidation of Invention 1, the violations of the support requirement referred to in 5. above have all been eliminated in Corrected Invention 1, as mentioned in (8) B. above. In addition, with regard to the other grounds for invalidation alleged by the Defendant in relation to Invention 1 and Corrected Invention 1, no ground for invalidation is found to exist at least in Corrected Invention 1 after the correction.

B. Regarding claims concerning Defendant's Production Process 1

According to A. above, without having to make determinations on other points, in regard to the claims based on Patent Right 1 concerning Defendant's Production Process 1, the requirements for re-defense of correction are fulfilled for Corrected Invention 1-1 (corresponding to Invention 1-1), Corrected Invention 1-2 (corresponding to Invention 1-2), Corrected Invention 1-3 (corresponding to Invention 1-4), and hence the defense of invalidity based on Article 104-3, paragraph (1) of the Patent Act is groundless for all the parts for which the re-defense of correction is allowed.

On the other hand, according to 5. (3) above, as the Plaintiff has not asserted re-defense of correction with regard to the claims based on Invention 1-3, the Plaintiff cannot exercise its rights against the Defendant based on the abovementioned defense of invalidity.

According to the above, the claims for which the Plaintiff can exercise its rights against the Defendant based on Patent Right 1 in relation to Defendant's Production Process 1, without being restricted by the defense of invalidity, are the claims based on Invention 1-1 (corresponding to Corrected Invention 1-1), Invention 1-2 (corresponding to Corrected Invention 1-2), and Invention 1-4 (corresponding to Corrected Invention 1-3).

C. Regarding claims concerning Defendant's Production Process 3

According to A. above, without having to make determinations on other points, in regard to the claims based on Patent Right 1 concerning Defendant's Production Process 3, the requirements for re-defense of correction are fulfilled for Corrected Invention 1-1, and hence the defense of invalidity based on Article 104-3, paragraph (1) of the Patent Act is groundless for the part for which the re-defense of correction is allowed.

On the other hand, as mentioned in A. above, because Defendant's Production Process 3 does not fall within the technical scope of Corrected Invention 1-3 (corresponding to Invention 1-4), re-defense of correction cannot be found to be established for the claims based on Invention 1-4. Accordingly, the Plaintiff cannot exercise its rights against the Defendant based on the abovementioned defense of invalidity with regard to the claims based on Invention 1-4 (corresponding to Corrected Invention 1-3).

According to the above, the claims for which the Plaintiff can exercise its rights against the Defendant based on Patent Right 1 in relation to Defendant's Production Process 3, without being restricted by the defense of invalidity, are the claims based on Invention 1-1 (corresponding to Corrected Invention 1-1).

7. Regarding Issue 6 (whether re-defense of correction of Patent 2 (Correction 2) can be established)

In light of the case, the ground for invalidation of the invention claimed in Patent 2 will be determined based on Issue 6.

(1) Whether Correction 2 satisfies the requirements for correction

A. Regarding correction concerning the number of amino acids (regarding Claim 1)

In Correction 2, the number of amino acids to be substituted, etc. in (ii) of Claim 1 was corrected from "one or several" before correction to "one to five," but it can be said that this correction is intended for restriction of the claims (Article 134-2, paragraph (1), item (i) of the Patent Act).

The Defendant asserts a violation of the requirements for correction (a violation of Article 126, paragraph (5) of the Patent Act) on the basis that Description 2 contains no statement on a numerical scope to make the number of amino acids to be substituted, etc. "one to five," but paragraph [0034] of Description 2 attached to the written application states a numerical scope as follows: "The yggB gene may be a gene having an amino acid sequence in which one or several amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 6, 62, 68, or 84 so far as the yggB gene enhances L-glutamic acid-producing ability of the coryneform bacterium. The number of 'several' as referred to herein means, for example, 2 to 20, preferably 2 to 10, and more preferably 2 to 5." Therefore, the abovementioned correction is a correction within the scope of the matters disclosed in the description, claims, or drawings attached to a written application, and complies with the requirement under Article 126, paragraph (5) of the Patent Act as applied mutatis mutandis pursuant to Article 134-2, paragraph (9) of that Act.

Furthermore, as this correction limits the number of amino acids to be substituted, etc. and does not substantially enlarge or alter the scope covered by the invention of Claim 1 before correction, it also complies with the requirement under Article 126, paragraph (6) of the Patent Act as applied mutatis mutandis pursuant to Article 134-2, paragraph (9) of that Act, and hence it satisfies all of the requirements for correction.

B. Regarding correction of the strain name (regarding paragraph [0033] of Description 2)

While Correction 2 makes a correction, in the part "The gene having a sequence of nucleotide numbers 1437 to 3035 of SEQ ID NO: 5 is a yggB gene of *Corynebacterium*

glutamicum ATCC13032 strain" in the third sentence in paragraph [0033] of Description 2 attached to the written application, to change "ATCC13032 strain" to "ATCC13869 strain" (Exhibits Ko 51 and 94), the Defendant alleges that this correction is not a correction of an error and thus impermissible.

First, in paragraph [0033] of Description 2 before correction, the nucleotide sequence of SEQ ID NO: 5 and the nucleotide sequence of SEQ ID NO: 83 are both indicated as being sequences derived from the ATCC13032 strain, and the sequences in the parts of these nucleotide sequences that correspond to the yggB gene partially do not coincide with each other (Exhibit Ko 4-2), so either of them is apparently an error.

In addition, while paragraph [0033] before correction has a statement "The gene having a sequence of nucleotide numbers 501 to 2099 of SEQ ID NO: 83 is encoded to nucleotide numbers 1336092 to 1337693 in the genome sequence registered as GenBank Accession No. NC_003450 of *Corynebacterium glutamicum* ATCC13032, and is registered as NCgl 1221 (NP_600492. Reports small-conductance ...[gi:19552490])," according to evidence (Exhibit Ko 94) and the entire import of oral arguments, it is found to be possible to confirm that SEQ ID NO: 83 is a sequence of the ATCC13032 strain by comparing information on the sequence of NCgl1221, which had been published as of the time of the filing of the application, and SEQ ID NO: 83.

Moreover, considering that paragraph [0103] before correction contains a statement indicating that the yggB gene of SEQ ID NO: 5 was derived from the "ATCC13869 strain," it is found that the statement in paragraph [0033] indicating that SEQ ID NO: 5 is derived from the "ATCC13032 strain" is an error, and correcting this to the "ATCC13869 strain" is found to constitute correction of an error or incorrect translation (Article 134-2, paragraph (1), item (ii) of the Patent Act).

In addition, in light of such contents of correction, this correction also complies with the requirements under Article 126, paragraphs (5) and (6) of the Patent Act as applied mutatis mutandis pursuant to Article 134-2, paragraph (9) of that Act, and satisfies all of the requirements for correction.

C. The Defendant has not specifically indicated a violation of the requirements for correction concerning Correction 2 apart from the corrections referred to in A. and B. above, and according to evidence (Exhibits Ko 51-1 and 94) and the entire import of oral arguments, all corrections in Correction 2 were made within the scope of the matters indicated in the description, claims, or drawings attached to the written application for the purpose of restriction of the claims (Article 134-2, paragraph (1), item (i) of the Patent Act), correction of errors or incorrect translations (item (ii) of that paragraph), or correction of a statement of claims which cites another statement of claims to a statement

which does not cite that other statement of claims (item (iv) of that paragraph), and they do not substantially enlarge or alter the claims; therefore, they satisfy the requirements for correction (Article 134-2, paragraph (9) and Article 126, paragraphs (5) and (6) of the Patent Act).

(2) Regarding whether the Defendant's Production Processes fall within the technical scope of Corrected Invention 2

A. Regarding Defendant's Production Processes 1 through 3

As examined in 3. above, the bacteria used in Defendant's Production Processes 1 through 3 are all *Corynebacterium glutamicum* into which a mutant-type yggB gene, wherein an A100T mutation has been added to the yggB gene having the amino acid sequence of SEQ ID NO: 6 and the amino acid sequence after the mutation is SEQ ID NO: 22, has been introduced, indicating an improvement in the glutamic acid-producing ability compared to a non-modified strain.

Consequently, when comparing Defendant's Production Processes 1 through 3 and Corrected Invention 2, the result is found to be as shown in Attachment 7-2 "Comparison between Defendant's Production Processes 1 through 3 and Corrected Invention 2." Accordingly, the bacteria used in Defendant's Production Processes 1 through 3 all fall within the technical scopes of Corrected Inventions 2-1, 2-2, 2-3, and 2-4, and Defendant's Production Processes 1 through 3, which use those bacteria, fall within the technical scope of Corrected Invention 2-5.

B. Regarding Defendant's Production Process 4

(A) As mentioned in 4. (5) A. above, Strains [xii] and [xiii] used in Defendant's Production Process 4 are both *Corynebacterium glutamicum* into which a mutant-type yggB gene, wherein an A98T mutation and a V241I mutation have been introduced into a yggB gene derived from *Corynebacterium callunae* (DSM20147 strain), has been introduced, indicating an improvement in the glutamic acid-producing ability compared to a non-modified strain in the presence of an excess of biotin.

Consequently, Strains [xii] and [xiii] used in Defendant's Production Process 4 fulfill Constituent Features 2-E'-1 and 2-E'-2 of Corrected Invention 2-2, but do not fulfill 2-D', and do not fulfill any of the constituent features of Corrected Invention 2-3. In addition, Defendant's Production Process 4 literally fulfills Constituent Feature 2-H' of Corrected Invention 2-5, except for the part citing Claims 1, 2, and 4 through 10, and fulfills Constituent Features 2-I' and 2-J.

(B) The configuration using the 19-type mutation is a configuration that is included in Corrected Invention 2-5 even after Correction 2, and it is a method of producing the glutamic acid in Claim 11 using a coryneform bacterium into which the mutant-type yggB

gene mentioned in (e) of Claim 6 citing Claim 4 after Correction 2, has been introduced.
(C) The differences between Defendant's Production Process 4 and the configuration using the 19-type mutation comprised in Defendant's Invention 2-5 lie in the parts relating to Constituent Features 2-D', 2-F-1, 2-F'-2, 2-F-3, and 2-H' (literally non-fulfilling parts mentioned in (A) above), and their specific contents are the same as Differences 1 through 3 mentioned in 4. (5) B. above.

Meanwhile, the circumstances concerning the first through fifth requirements of the doctrine of equivalents with regard to Defendant's Production Process 4 using Strains [xii] and [xiii] and the configuration using the 19-type mutation comprised in Corrected Invention 2-5 are the same as those mentioned in 4. (6) through (10) above, and no circumstances that would overturn the conclusions regarding the respective requirements are found in the evidence for the present case.

Consequently, it is reasonable to construe that Defendant's Production Process 4 using Strains [xii] and [xiii] falls within the technical scope of Corrected Invention 2-5 as being an equivalent to the configuration using the 19-type mutation indicated in the claims.

(3) Whether or not Corrected Invention 2 lacks novelty based on Exhibit Otsu 42 Invention

A. Statements in Exhibit Otsu 42 Document

The contents described in Exhibit Otsu 42 Document include the following (Exhibit Otsu 42, the entire import of oral arguments).

(A) Paragraph [0003]

"For example, *Corynebacterium glutamicum* is a Gram-positive bacterium identified as a glutamic acid-producing bacterium, and many amino acids are produced by mutants thereof. For example, 1,000,000 ton/year of L-glutamic acid which is useful as a seasoning for umami (delicious taste), 250,000 ton/year of L-lysine which is a valuable additive for livestock feeds and the like, and several hundred ton/year or more of other amino acids, such as L-arginine, L-proline, L-glutamine, L-tryptophan, and the like, have been produced in the world (*Nikkei Bio Yearbook 99*, published by Nikkei BP (1998))."

(B) Paragraph [0009]

"An object of the present invention is to provide a polynucleotide and a polypeptide derived from a microorganism of coryneform bacteria which are industrially useful, sequence information of the polynucleotide and the polypeptide, a method for analyzing the microorganism, an apparatus and a system for use in the analysis, and a method for breeding the microorganism."

(C) Paragraph [0010]

"The present invention provides a polynucleotide and an oligonucleotide derived from

a microorganism belonging to coryneform bacteria, ... a polypeptide encoded by the polynucleotide ..."

(D) Table 1 (Lines 1 through 2 on page 110)

The following sequence: SEQ NO (DNA) "1401": SEQ NO (a. a.) "4901"; Initial (nt) "1337567"; Terminal (nt) "1336095"; ORF (bp) "1473"; db Match "sp: YILV__CORGL"; Homologous gene "Corynebacterium glutamicum ATCC13032 yilV"; Identity (%) "100.0"; Similarity (%) "100.0"; Matched length (a. a.) "62"; Function "hypothetical membrane protein."

B. According to evidence (Exhibit Ko 4-2, Exhibits Otsu 42 and 43) and the entire import of oral arguments, the sequence mentioned in A. (D) above, which is described in Exhibit Otsu 42 Document, is the sequence of the polypeptide of SEQ ID NO: 4901 encoded by the gene of SEQ ID NO: 1401, and this polypeptide sequence is found to correspond to the amino acid sequence of SEQ ID NO: 84 consisting of 533 amino acids mentioned in Description 2, which is a sequence of a yggB gene derived from *Corynebacterium glutamicum*, excluding the amino acids at positions 1 through 42.

C. The Defendant alleges that the sequence of SEQ ID NO: 4901 in Exhibit Otsu 42 Document discloses the amino acid sequence described in (ii) of Claim 1 of Patent 2 (the statement after Correction 2 is as follows: "(ii) substitution, deletion, or insertion of one to five amino acids in a region selected from the group consisting of amino acid numbers 1 to 23, 86 to 108, and 110 to 132 of an amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.").

However, as mentioned in B. above, the sequence of SEQ ID NO: 4901 in Exhibit Otsu 42 Document has excluded all amino acids at positions 1 through 42 from the amino acid sequence of SEQ ID NO: 84 in Description 2, and therefore it does not constitute the abovementioned amino acid sequence in (ii) of Claim 1 after Correction 2.

Consequently, without having to make determinations on other points, it cannot be said that Corrected Invention 2-5 citing Claim 1 or Claim 10 includes the same invention as Exhibit Otsu 42 Invention, and hence a ground for invalidation, i.e. lack of novelty, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (1), item (iii) of the Patent Act) based on Exhibit Otsu 42 Invention does not exist with regard to Corrected Invention 2-5.

(4) Whether or not Corrected Invention 2 lacks an inventive step based on Exhibit Otsu 24 Invention as the primary cited document

A. Regarding Exhibit Otsu 24 Invention

The contents described in Exhibit Otsu 24 Document are as mentioned in 4. (2) D. (A) above, and as examined in 4. (2) D. (B) above, although Exhibit Otsu 24 Document

suggests that *Corynebacterium glutamicum* has osmoregulated channels having similar characteristics as mechanosensitive channels of *E. coli*, it does not show that the osmoregulated channels contribute to efflux of glutamic acid. Rather, it states that the channels were unrelated to efflux of glutamic acid, and derived a conclusion that efflux of glutamic acid is mediated not by osmoregulated channels, but by a carrier system as had been indicated before.

Consequently, it is reasonable to find that Exhibit Otsu 24 Document discloses an invention of "*Corynebacterium glutamicum* having osmoregulated channels for which similarity with mechanosensitive channels of *E. coli* is suggested" (Exhibit Otsu 24 Invention), and the Defendant's allegation that Exhibit Otsu 24 Invention discloses "*Corynebacterium glutamicum* having osmoregulated channels, related to efflux of glutamic acid" cannot be adopted.

B. Regarding involvement of an inventive step in Corrected Invention 2-5 citing Claim 1

(A) Comparison between Exhibit Otsu 24 Invention and Corrected Invention 2-5 citing Claim 1

The following are found as a result of comparing Exhibit Otsu 24 Invention with Corrected Invention 2-5 citing Claim 1.

a. Common feature

The bacteria mentioned in Exhibit Otsu 24 Invention and Claim 1 are both coryneform bacteria having osmoregulated channels.

b. Differences

Exhibit Otsu 24 Invention and Corrected Invention 2-5 citing Claim 1 differ in the following respects.

Difference 1: Corrected Invention 2-5 is an invention of a method of producing glutamic acid comprising the steps of cultivating a coryneform bacterium in a medium, to allow glutamic acid to be produced and accumulated in the medium or within the bacterial cell, and collecting it from the medium or the bacterial cell, whereas Exhibit Otsu 24 Invention is an invention of a coryneform bacterium.

Difference 2: In Corrected Invention 2-5, a mutant-type yggB gene is introduced into the coryneform bacterium, and this mutant-type yggB gene is one into which any of the mutations mentioned in (i), (i'), (i''), or (ii) of Claim 1 has been introduced, indicating an improvement in the glutamic acid-producing ability compared to a non-modified strain, whereas in the coryneform bacterium of Exhibit Otsu 24 Invention, the yggB gene is not specified, and thus the glutamic acid-producing ability is not specified.

(B) Ease in conceiving of Difference 1

There is no dispute between the parties concerning the fact that a person skilled in the

art could have easily conceived of Difference 1 as of Priority Date 2.

(C) Ease in conceiving of Difference 2

According to the results of the examination made in 4. (2) D. above, the fact that osmoregulated channels contribute to efflux of glutamic acid in coryneform bacteria including *Corynebacterium glutamicum* is not found to have been well-known to persons skilled in the art as of Priority Date 2, and Exhibit Otsu 24 Document also does not state that osmoregulated channels contribute to efflux of glutamic acid. Therefore, it had been concluded that efflux of glutamic acid is mediated not by osmoregulated channels, but by a carrier system.

The Defendant alleges that a person skilled in the art could have easily conceived of adopting the configuration relating to Difference 2 by applying Exhibit Otsu 26 Document, Exhibit Otsu 29 Document, Exhibit Otsu 30 Invention, Exhibit Otsu 31 Invention, and Exhibit Otsu 41 Document as well as common general technical knowledge to Exhibit Otsu 24 Invention. However, the abovementioned documents and inventions contain no statements that suggest efflux of glutamic acid from osmoregulated channels in coryneform bacteria (Exhibits Otsu 26, 29 through 31, and 41), and as mentioned above, such well-known art or common general technical knowledge is not found to have existed as of Priority Date 2.

If so, it should be said that there is no motivation in Exhibit Otsu 24 Invention for a person skilled in the art to pay attention to the yggB gene that encodes osmoregulated channels in coryneform bacteria by applying the abovementioned documents and inventions as well as common general technical knowledge; thus, a person skilled in the art could not have easily conceived of the configuration relating to Difference 2 as of Priority Date 2.

(C) Accordingly, it cannot be said that a person skilled in the art could have easily conceived of Corrected Invention 2-5 citing Claim 1 based on Exhibit Otsu 24 Invention as the primary cited document.

C. Regarding involvement of an inventive step in Corrected Invention 2-5 citing Claim 4, Claim 6, or Claim 10

Claim 4 after Correction 2 is an invention of a coryneform bacterium into which a mutant-type yggB gene having a mutation that is included in the mutation mentioned in (ii) of Claim 1 has been introduced, while Claim 6 and Claim 10 after Correction 2 are inventions of coryneform bacteria that have further restricted Claim 1. Therefore, according to the results of the examination made in A. above, similar to the case of Corrected Invention 2 citing Claim 1, it cannot be said that a person skilled in the art could have easily conceived of any of Corrected Invention 2-5 citing Claim 4, Claim 6,

or Claim 10 based on Exhibit Otsu 24 Invention as the primary cited document.
D. Consequently, a ground for invalidation, i.e. lack of an inventive step, (Article 123, paragraph (1), item (ii) and Article 29, paragraph (2) of the Patent Act) based on Exhibit Otsu 24 Invention as the primary cited document is not found for Corrected Invention 2-5 citing Claim 1, Claim 4, Claim 6, or Claim 10.
(5) Whether or not Corrected Invention 2 violates the enablement requirement or the support requirement
A. Regarding culture conditions
(A) Regarding Working Example 8 and Working Example 10

As mentioned in 4. (3) B. (B) above, Working Example 8 indicates that under a condition with an excess of biotin (a non-inducing condition), the glutamic acid production increases as compared to a non-modified strain due to the configuration using the 19-type mutation included in Corrected Invention 2-5, and the experiment in Exhibit Otsu 44 performed by the Defendant does not overturn the credibility of the experiment results in Working Example 8.

In addition, as mentioned in 4. (3) B. (A) above, although Working Example 10 indicates that the glutamic acid production increases under an inducing condition due to the configuration using the 19-type mutation, it does not indicate that such effect is only observed under an inducing condition (paragraphs [0125] through [0128]).
(B) Regarding a violation of the support requirement

The problem to be solved by Corrected Invention 2-5 is "to provide novel art to improve the L-glutamic acid-producing ability of a coryneform bacterium when L-glutamic acid is produced by using the coryneform bacterium" (paragraph [0007]).

As mentioned in (A) above, it can be said that Working Examples 8 and 10 disclose that the configuration using the 19-type mutation included in Corrected Invention 2-5 improves the glutamic acid-producing ability not only under an inducing condition, but also under a non-inducing condition, and there is no other evidence sufficient to find that the abovementioned problem can be solved only under an inducing condition.

Consequently, it cannot be said, based on the fact that use of an inducing condition as the culture condition is not required, that Corrected Invention 2-5 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention and the common general technical knowledge as of the time of the filing of the application. Therefore, the Defendant's allegation of a violation of the support requirement relating to this point is groundless.
(C) Regarding a violation of the enablement requirement

According to the results of the examination made in 4. (3) B. above, it can be said that a person skilled in the art can work the invention relating to the configuration using the 19-type mutation without undergoing excessive trial and error based on the statements on culture conditions in Description 2 and common general technical knowledge as of the time of the filing of the application, not only under an inducing condition, but also under a non-inducing condition.

Also with regard to other configurations included in Corrected Invention 2-5, no circumstances are found in terms of culture conditions which suggest that a person skilled in the art cannot achieve those configurations under a non-inducing condition based on the statements on culture conditions in Description 2 and common general technical knowledge as of the time of the filing of the application, and hence the Defendant's allegation of a violation of the enablement requirement relating to this point is groundless.

B. Regarding the mutations mentioned in (i) and (i') of Claim 1

(A) According to the results of the examination made in 4. (2) above, the technical idea of Corrected Invention 2 is found to be to promote efflux of glutamic acid and improve the glutamic acid-producing ability by focusing on the fact that the YggB protein, which is an osmoregulated channel encoded by the yggB gene of a coryneform bacterium, is involved in efflux of glutamic acid, and by modifying the YggB protein by using a mutant-type yggB gene into which a C-terminal side mutation or mutations in transmembrane regions are introduced. Meanwhile, the mutations mentioned in (i) and (i') of Claim 1 introduce a mutation into the part of the mutant-type yggB gene that encodes the C-terminal side region (paragraph [0070]).

(B) Description 2 provides an explanation on the C-terminal side mutation in general in paragraph [0070], and as its example, it describes in paragraph [0071] a 2A-1-type mutation wherein the amino acid sequence in a region positioned downstream from position 419 in the C-terminal side of the amino acid sequence (positions 419 through 533 of the amino acid sequence of SEQ ID NO: 6) is substituted with a sequence consisting of five amino acids which is derived from a short insertion sequence, and states that the 2A-1-type mutation includes a mutation wherein the region positioned downstream from position 419 in the C-terminal side of SEQ ID NOs: 6, 62, 68, 84 and 85 is deleted or substituted. Further, Working Examples 5 and 6 in Description 2 state that it was confirmed that a glutamic acid-producing ability was improved in the 2A-1-type mutation wherein the region positioned downstream from position 419 in the C-terminal side is substituted with five amino acids (paragraphs [0107] through [0113]).

(C) Based on the basic technical idea mentioned in (A) above, it is found that a person skilled in the art who read the statements in Description 2 referred to in (B) above would

recognize that, with regard to the mutations mentioned in (i) and (i') of Claim 1, the three-dimensional structure of the YggB protein would be modified by introduction of a mutation such as deletion or substitution, in a region positioned downstream from position 419 in the C-terminal side, to thereby improve the glutamic acid-producing ability.

Although the Defendant alleges that Description 2 contains no statements on a coryneform bacterium into which the mutation mentioned in (i) of Claim 1 was introduced, as mentioned above, the 2A-1-type mutation that was introduced in Working Examples 5 and 6 is a mutation wherein 115 amino acids at positions 419 through 533 in the C-terminal side region have been substituted with five amino acids, and in view of the embodiment of the mutation, it can be said that a person skilled in the art can recognize that even if the region positioned downstream from position 419 in the C-terminal side region is deleted, the three-dimensional structure of the YggB protein in the part encoded by amino acids in the C-terminal side region would be similarly modified, and the glutamic acid-producing ability would be improved.

In addition, the Defendant alleges that it is not possible to understand that the mutations in (i') of Claim 1 other than the 2A-1-type mutation mentioned in Working Examples 5 and 6 also have the same effect. However, as mentioned above, it can be said that a person skilled in the art can recognize, based on the statements in Description 2, the basic principle that the glutamic acid-producing ability is improved due to a change in the three-dimensional structure of the YggB protein caused by, for example, insertion of an insertion sequence to the region positioned downstream from position 419 in the C-terminal side.

Consequently, with regard to coryneform bacteria of which glutamic acid-producing ability has been improved by introduction of the mutations mentioned in (i) and (i') of Claim 1, it cannot be said that Corrected Invention 2-5 citing Claim 1 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention. Therefore, the Defendant's allegation of a violation of the support requirement relating to this point is groundless. In addition, it can be said that a person skilled in the art can obtain the abovementioned coryneform bacteria of which glutamic acid-producing ability has been improved and work Corrected Invention 2-5 without undergoing excessive trial and error based on the abovementioned statements in Description 2, and hence, it cannot be said that the invention fails to fulfill the enablement requirement.

C. Regarding the mutation mentioned in (i") of Claim 1

(A) Paragraph [0072] of Description 2 discloses 12 substitutable prolines in the region

positioned downstream from position 419 in the C-terminal side of the yggB gene, and that it is considered that these prolines play an important role in maintaining the three-dimensional structure of the YggB protein. In addition, Working Examples 12 and 13 in Description 2 state that a mutant strain was produced wherein proline at position 424 in the amino acid sequence of SEQ ID NO: 68 was substituted with leucine (66-type mutation), and proline at position 437 in the amino acid sequence of SEQ ID NO: 6 was substituted with serine (22-type mutation), and that the 22-type mutant strain mentioned in Working Example 13 actually improved the glutamic acid-producing ability (paragraphs [0130] through [0138]).

Moreover, according to evidence (Exhibits Ko 44 through 46) and the entire import of oral arguments, it is found to have been well-known to persons skilled in the art that proline is generally an amino acid having a special property which is involved in the formation of the three-dimensional structure of a protein.

(B) It can be said that, based on the statements in Description 2 mentioned in (A) above and common general technical knowledge concerning the property of proline, as well as the basic technical idea of Corrected Invention 2 mentioned in B. (A) above, a person skilled in the art can recognize that, by substituting the proline in the region positioned downstream from position 419 in the C-terminal side with another amino acid, the three-dimensional structure of the YggB protein in the part encoded by amino acids in that region would be modified, and the glutamic acid-producing ability would be improved.

Consequently, with regard to coryneform bacteria of which glutamic acid-producing ability has been improved by introduction of the mutation mentioned in (i") of Claim 1, it cannot be said that Corrected Invention 2-5 citing Claim 1 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention. In addition, it can be said that a person skilled in the art can obtain the abovementioned coryneform bacteria of which glutamic acid-producing ability has been improved and work Corrected Invention 2-5 without undergoing excessive trial and error based on the abovementioned statements in Description 2. Therefore, the invention is not found to violate the support requirement or the enablement requirement in relation to this point.

The Defendant indicates among other matters that while many prolines could be subject to substitution in the mutation mentioned in (i") of Claim 1, the glutamic acid productivity has been actually confirmed only for the 22-type mutation mentioned in Working Example 13, and that the result could differ if proline at a different position was substituted from among the abovementioned prolines. However, given that the basic

principle has been clarified and that the substitutable prolines are specifically disclosed in Description 2 as mentioned above, even by taking the Defendant's allegation into consideration, the invention is not found to violate the support requirement or the enablement requirement, as mentioned above.

D. Regarding the mutation mentioned in (ii) of Claim 1 (a mutation in the transmembrane region)

(A) The mutation mentioned in (ii) of Claim 1 is a mutation to be introduced into the yggB gene wherein one to five amino acids are substituted, deleted, or inserted in the transmembrane region.

Paragraph [0073] of Description 2 states, as generalities concerning mutations in the transmembrane region, that the YggB protein is presumed to have five transmembrane regions, that these transmembrane regions correspond to amino acid numbers 1 to 23 (the first transmembrane region), amino acid numbers 86 to 108 (the fourth transmembrane region), and amino acid numbers 110 to 132 (the fifth transmembrane region) in the amino acid sequences of SEQ ID NOs: 6, 62, 68, 84, and 85, which are stated in (ii) of Claim 1 as regions into which mutations should be introduced, as well as the details of the mutations to be introduced into the transmembrane regions.

As specific examples of the mutations, Description 2 mentions the A1-type mutation as a mutation in the first transmembrane region, the 19-type mutation as a mutation in the fourth transmembrane region, and the L30-type mutation and the 8-type mutation as examples of mutations in the fifth transmembrane region (paragraphs [0074] through [0076]). Moreover, Description 2 states the following: as Working Example 7, a method of producing a strain into which the A1-type mutation has been introduced and the fact that the glutamic acid-producing ability has been improved in this strain (paragraphs [0114] through [0118]); as Working Examples 8 and 10, a method of producing a strain into which the 19-type mutation has been introduced and the fact that the glutamic acid-producing ability has been improved in this strain (paragraphs [0119] through [0121] and [0125] through [0128]); as Working Example 9, a method of producing a strain into which the L30-type mutation has been introduced and the fact that the glutamic acid-producing ability has been improved in this strain (paragraphs [0122] through [0124]); and as Working Example 11, a method of producing a strain into which the 8-type mutation has been introduced (paragraph [0129]).

(B) It can be said that, based on the statements in Description 2 mentioned in (A) above and the basic technical idea of Corrected Invention 2 mentioned in B. (A) above, a person skilled in the art can recognize the basic principle for solving the problem, which is adding modification to the transmembrane region of the YggB protein which forms the

osmoregulated channel relating to efflux of glutamic acid to promote efflux of glutamic acid and improve the glutamic acid-producing ability.

Considering that Description 2 discloses working examples in which the glutamic acid-producing ability was improved for three transmembrane regions, and that (ii) of Claim 1 restricts the regions into which the mutation should be introduced and also restricts the number of amino acids to be substituted, deleted, or inserted to one to five, with regard to coryneform bacteria of which glutamic acid-producing ability has been improved by introduction of the mutation mentioned in (ii) of Claim 1, it cannot be said that Corrected Invention 2-5 citing Claim 1 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention. In addition, it can be said that a person skilled in the art can obtain the abovementioned coryneform bacteria of which glutamic acid-producing ability has been improved and work Corrected Invention 2-5 without undergoing excessive trial and error based on the abovementioned statements in Description 2. Therefore, the invention is not found to violate the support requirement or the enablement requirement in relation to this point.

E. Regarding SEQ ID NO: 85

(A) The mutations mentioned in (i), (i'), (i''), or (ii) of Claim 1 include not only those that use the amino acid sequences of SEQ ID NOs: 6, 62, 68, and 84, but also those that use the amino acid sequence of SEQ ID NO: 85 of the yggB gene before mutation.

Paragraph [0034] of Description 2 states that the amino acid sequence of the yggB gene into which a mutation is to be introduced may be a gene having an amino acid sequence in which one or several amino acids are substituted, deleted, etc. in an amino acid sequence of SEQ ID NO: 6, 62, 68, or 84, and that, in the case of substitution, it should preferably be a conservative substitution, while showing specific examples of conservative substitution of the respective types of amino acids. Then, paragraph [0035] discloses SEQ ID NO: 85 as a sequence wherein 14 locations of amino acids in the amino acid sequence of SEQ ID NO: 6 that may be substituted or deleted are indicated by Xaa. In addition, Description 2 also has statements on conservative substitution in paragraphs [0078] and [0079].

Furthermore, according to evidence (Exhibit Ko 48), it is found to have been well-known to persons skilled in the art as of the time of the filing of the application that, as a property of protein, the functions of the protein are maintained even if substitution between amino acids having similar size and similar polarity (conservative substitution) occurs at a site other than an active center of a protein or a site of functional importance in the protein.

(B) In light of the statements in Description 2 mentioned in (A) above and common general technical knowledge as of the time of the filing of the application, a person skilled in the art can recognize that the locations indicated by Xaa in SEQ ID NO: 85 are sites that are not of functional importance in the protein and that may be substituted or deleted as mentioned above, and recognize a certain scope of substitutions that are permissible, based on the abovementioned significance of conservative substitution. If so, it can be said that even if Description 2 contains no working example in which a mutation has been added to the amino acid sequence of SEQ ID NO: 85, given the points examined in B. through D. above, a person skilled in the art can recognize that the glutamic acid-producing ability can also be improved by introducing the respective mutations mentioned in Claim 1 into the amino acid sequence of SEQ ID NO: 85, similar to the case of the amino acid sequences of the other SEQ ID Nos listed in Claim 1.

Considering that the amino acids that can be substituted, etc. from SEQ ID NO: 6 (Xaa) are concretely specified for SEQ ID NO: 85, and details of desirable substitutions are disclosed in Description 2 as mentioned in (A) above, with regard to coryneform bacteria of which glutamic acid-producing ability has been improved by introduction of the respective mutations mentioned in Claim 1 into the amino acid sequence of SEQ ID NO: 85 as well, it cannot be said that Corrected Invention 2-5 citing Claim 1 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention and common general technical knowledge as of Priority Date 2. In addition, it can be said that a person skilled in the art can obtain the abovementioned coryneform bacteria of which glutamic acid-producing ability has been improved and work Corrected Invention 2-5 without undergoing excessive trial and error based on the abovementioned statements in Description 2 and common general technical knowledge as of the time of the filing of the application. Therefore, the invention is not found to violate the support requirement or the enablement requirement in relation to this point.

F. Regarding the mutation mentioned in Claim 4

The mutations to be introduced in Claim 4 after Correction 2 are included in the mutations mentioned in (ii) of Claim 1.

In addition to the point examined in D. above, with regard to Claim 4, Correction 2 further restricts the amino acids to be substituted from alanine at position 100 or 111. Given that the 19-type mutation mentioned in Working Examples 8 and 10 is disclosed with regard to a mutation that substitutes alanine at position 100 with threonine, the 8-type mutation mentioned in Working Example 11 is disclosed with regard to a mutation that substitutes alanine at position 111 with threonine, and the L30-type mutation

mentioned in Working Example 9 with regard to a mutation that substitutes alanine at position 111 with valine, all of which are mutations included in Claim 4, Corrected Invention 2-5 citing Claim 4 is also not found to violate the support requirement or the enablement requirement. According to the results of the examination made in E. above, the same applies to the part of the mutation mentioned in Claim 4 which introduces a mutation into the amino acid sequence of SEQ ID NO: 85.

G. Regarding the mutation mentioned in Claim 6

(A) The amino acid sequences of SEQ ID NOs: 8, 20, 22, 24, 64, 70, and 74 mentioned in (a), (c), (e), (g), (i), (k), and (m) of Claim 6 respectively correspond to the amino acid sequences encoded by the yggB gene after the 2A-1-type mutation mentioned in Working Example 6, the A1-type mutation mentioned in Working Example 7, the 19-type mutation mentioned in Working Examples 8 and 10, the L30-type mutation mentioned in Working Example 9, the 8-type mutation mentioned in Working Example 11, the 66-type mutation mentioned in Working Example 12, and the 22-type mutation mentioned in Working Example 13.

The Defendant alleges that, with regard to the mutant-type yggB genes mentioned in (b), (d), (f), (h), (j), (l), and (n) of Claim 6 wherein deletion, etc. of one to five amino acids has been further added to the respective amino acid sequences after these mutations, it is not possible to understand what kind of mutant protein would improve the glutamic acid productivity in a medium containing an excess of biotin as a result of adding substitution, etc. to one to five amino acids at arbitrary positions.

However, as mentioned in E. (A) above, it is found to have been well-known to persons skilled in the art as of the time of the filing of the application that, as a property of protein, the functions of the protein are maintained even if substitution between amino acids having similar size and similar polarity (conservative substitution) occurs at a site other than an active center of a protein or a site of functional importance in the protein. In addition, paragraphs [0078] and [0079] of Description 2 state that a mutation that may be added to the amino acid sequence of SEQ ID NO: 8, 20, 22, 24, 64, 70, or 74 is preferably conservative substitution, while showing specific examples of conservative substitution that may be added to these amino acid sequences.

(B) Considering the statements in Description 2 mentioned in (A) above and common general technical knowledge, the points examined in B. through D. above, as well as the fact that, regarding the mutant-type yggB genes mentioned in (b), (d), (f), (h), (j), (l), and (n) of Claim 6, the number of amino acids that may be substituted from the amino acid sequence of SEQ ID NO: 8, 20, 22, 24, 64, 70, or 74 are restricted to one to five, with regard to coryneform bacteria of which glutamic acid-producing ability in the case of

being cultured in a medium containing an excess of biotin has been improved by introduction of the respective mutant-type yggB genes mentioned above, it cannot be said that Corrected Invention 2-5 citing Claim 6 falls beyond the scope in which a person skilled in the art can recognize that the problem to be solved by the invention can be solved in light of the statements in the detailed explanation of the invention and common general technical knowledge as of the time of the filing of the application. In addition, it can be said that a person skilled in the art can obtain the abovementioned coryneform bacteria of which glutamic acid-producing ability in the case of being cultured in a medium containing an excess of biotin has been improved and work Corrected Invention 2-5 without undergoing excessive trial and error based on the abovementioned statements in Description 2 and common general technical knowledge as of Priority Date 2. Therefore, the invention is not found to violate the support requirement or the enablement requirement in relation to this point.

H. Regarding the mutation mentioned in Claim 10

Claim 10 adds a restriction so that the coryneform bacteria into which the mutant-type yggB genes mentioned in Claims 1, 4, and 6 are to be introduced are limited to bacteria belonging to the genus *Corynebacterium* or the genus *Brevibacterium*.

Paragraphs [0012] and [0013] of Description 2 state that the coryneform bacteria covered by the invention include, in addition to a bacterium belonging to the genus *Corynebacterium*, a bacterium which has been conventionally classified into the genus *Brevibacterium* but is currently classified into the genus *Corynebacterium*, and also *Brevibacterium* bacteria which are very closely related to the genus *Corynebacterium* bacteria, and indicate specific examples of these bacteria. In addition, the working examples also contain examples in which a mutant-type yggB gene has been introduced into *Corynebacterium glutamicum* and *Brevibacterium flavum*, both of which are currently classified into the genus *Corynebacterium* (paragraph [0119] of Working Example 8, paragraph [0129] of Working Example 11, etc.).

According to these statements in Description 2 and the results of the examination made in B. through G. above, Corrected Invention 2-5 citing Claim 10 is also not found to violate the support requirement or the enablement requirement.

I. Regarding the method of producing and collecting glutamic acid (Claim 11)

The Defendant alleges, with regard to Invention 2-5 before correction, that Description 2 contains no statements about allowing glutamic acid to be produced in the medium and about collecting glutamic acid from the bacterial cell, and that the invention therefore violates the enablement requirement and the support requirement in relation to this point. However, the Defendant has not asserted a ground for invalidation in relation

to this point regarding Corrected Invention 2-5.

In Claim 11 after Correction 2, the statement about collecting glutamic acid from the bacterial cell has been deleted, so violation of the enablement requirement and the support requirement in relation to this point does not present a problem with regard to Corrected Invention 2-5. Meanwhile, as to the point to cultivate a coryneform bacterium in a medium and "to allow L-glutamic acid to be produced and accumulated in the medium" (Claim 11 after Correction 2), according to the statements in paragraphs [0085] through [0090] of Description 2 and evidence (Exhibits Ko 8 and 9, Exhibit Otsu 2) as well as the entire import of oral arguments, it is found to have been a well-known matter for persons skilled in the art as of Priority Date 2.

Consequently, Corrected Invention 2-5 is not found to violate the enablement requirement or the support requirement with regard to the method of producing and collecting glutamic acid.

(6) Whether or not Corrected Invention 2 violates the clarity requirement

The Defendant alleges, with regard to "an excess of biotin" mentioned in Claim 6 of Patent 2, that the amount intended by the phrase "an excess" is unknown.

However, paragraph [0032] of Description 2 states "'a condition with an excess of biotin' means, for example, a condition in which the medium contains biotin in an amount of 30 μg/L or more, preferably 40 μg/L, and more preferably 50 μg/L." As it is possible to understand the meaning and details of "an excess of biotin" from the statements in Description 2, the statements on this point do not violate the clarity requirement (Article 36, paragraph (6), item (ii) of the Patent Act).

Consequently, Corrected Invention 2-5 citing Claim 6 does not contain the ground for invalidation, i.e. a violation of the clarity requirement.

(7) Summary on the defense of invalidity concerning Patent Right 2

As mentioned above, Correction 2 satisfies the requirements for correction, and the Defendant's Production Processes all fall within the technical scope of Corrected Invention 2-5.

Moreover, with regard to the respective grounds for invalidation asserted by the Defendant with regard to Invention 2-5 and Corrected Invention 2-5, no ground for invalidation is found to exist at least for Corrected Invention 2-5 after correction. Thus, all of the requirements for re-defense of correction are satisfied, and therefore, without having to make determinations on other points, all of the Defendant's defenses of invalidity based on Article 104-3, paragraph (1) of the Patent Act against claims based on Patent Right 2 are groundless.

8. Regarding Issue 8 (occurrence or non-occurrence and the value of damages)

(1) Organization of the periods of use of the Defendant's Production Processes and the correspondence with the Patent Rights

A. As mentioned in 1. (4) above, with regard to use or non-use of the Defendant's Production Processes in each period during the subject period, Defendant's Production Process 1 was used in the periods of use of Strains [ii] and [x] described in Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question," and the processes used in the other periods are as described in the "Plaintiff's alternative claims" column in that Attachment.

Consequently, the periods of use of the Defendant's Production Processes can be organized as follows.

(A) Defendant's Production Process 1

From January 2011 through May 2014

From August 2015 through June 2016

(B) Defendant's Production Process 2

From April through June, 2014, and November and December of that year

June 2015

From May through August, 2016

(C) Defendant's Production Process 3

From June 2014 through November 2015

(D) Defendant's Production Process 4

From July 2016 through December 2017

B. According to 2., 5. (3), and 6. (9) above, the Defendant's Production Processes against which claims can be filed based on Patent Right 1 are Defendant's Production Processes 1 and 3, whereas according to 3., 4. (11), and 7. (7) above, claims based on Patent Right 2 can be filed against all of the Defendant's Production Processes.

If so, the relation of the Patent Rights with the Defendant's Production Processes against which rights can be exercised based thereon and with the Defendant's Products produced by those processes can be organized in the order of the time of production as follows. The MSG in question produced during the period from 2011 through 2017 (hereinafter referred to as the "period of use of the Defendant's Production Processes") represents products produced by any of the Defendant's Production Processes (the Defendant's Products), and rights can be exercised against them based on Patent Right 1 or 2.

(A) January 2011 through August 22, 2013

Defendant's Production Process 1 was used during this period. As this was before registration of establishment of Patent Right 2, rights can be exercised based on Patent

Right 1 against Defendant's Product 1.

(B) August 23, 2013 (the date of registration of establishment of Patent Right 2) through June 2016

Defendant's Production Process 1 or 3 was used throughout this period. Rights can be exercised based on Patent Rights 1 and 2 against Defendant's Product 1 or 3 produced by either of these processes.

Defendant's Production Process 2 was additionally used during a part of this period. Rights can be exercised based on Patent Right 2 against Defendant's Product 2 produced by this process.

(C) July 2016 through December 2017

Defendant's Production Process 2 or 4 was used throughout this period. Rights can be exercised based on Patent Right 2 against Defendant's Product 2 or 4 produced by either of these processes.

(2) Scope of working of inventions for which the Defendant is liable for tort

A. Finding of facts

According to the basic facts, etc. in No. 2, 2 above, the evidence shown below, and the entire import of oral arguments, the following facts are found with regard to production of the MSG in question and the sale thereof to Japanese customers during the period of use of the Defendant's Production Processes.

(A) Relationship of the CJ Group

The Defendant and CJ Indonesia belong to the CJ Group, CJ Indonesia is a wholly owned subsidiary company of CJCJ, the central company of the CJ Group, and the Defendant is a wholly owned subsidiary company of CJ Company, a holding company, holding 44% of CJCJ's shares (No. 2, 2. (1) B. above).

In the CJ Group, the group companies share roles in producing and selling CJ-brand and other products in countries around the world. According to the website of the bio section of the CJ Group, the section's production bases are located in Asia, North America, and South America, and the factory of CJ Indonesia in Indonesia is introduced as one of the section's production bases, but the section has no production base in Japan. The website indicates that the section's sales bases are located in Asia, Europe, North America, South America, the Middle East, and North Africa and that CJCJ in South Korea is the headquarters, and introduces CJ Indonesia in Jakarta and the Defendant in Tokyo as some of the section's sales bases in Asia (Exhibit Ko 97).

CJCJ has a research and development section, and the strain used for the production of MSG in question in CJ Indonesia's factory in Indonesia during the period of use of the Defendant's Production Processes was a strain that had been developed by CJCJ and sent

to CJ Indonesia (Exhibits Otsu 1, 2, and 5).

(B) Portion sold by the Defendant

During the period of use of the Defendant's Production Processes, the Defendant purchased, from CJ Indonesia, the MSG in question that had been produced in Indonesia by CJ Indonesia, imported it into Japan from Indonesia, and both offered to transfer it and transferred it in Japan (the portion sold by the Defendant in No. 2, 2. (7) A. above; Exhibits Otsu 101, 104, 111, and 113).

(C) Portion sold by CJ Indonesia

During the period of use of the Defendant's Production Processes, CJ Indonesia sold the MSG in question which it had produced by itself in Indonesia to Japanese customers as a seller, separately from the portion sold by the Defendant (the portion sold by CJ Indonesia in No. 2, 2. (7) A. above; Exhibit Otsu 101). The specific mode of this transaction was as follows.

a. Upon conclusion of sales contracts for the portion to be sold by CJ Indonesia, the order forms from Japanese customers contained a statement that the seller of the MSG in question was CJ Indonesia (Exhibits Otsu 102-1 and 103-1).

However, an order form to be sent to CJ Indonesia was sometimes sent to the Defendant by a Japanese customer, and in such case, the Defendant sent that order form to CJ Indonesia (Exhibit Otsu 103-1).

b. With regard to the portion to be sold by CJ Indonesia, the MSG in question was shipped in Indonesia and transported to Japan with CJ Indonesia as the shipper. The invoices and bills of lading, etc. relating to that transportation also stated that the shipper was CJ Indonesia.

Upon transportation of the MSG in question to Japan, the invoices relating to the transportation stated that the terms and conditions between CJ Indonesia and Japanese customers were to be cost, insurance, and freight (CIF) or cost and freight (CFR) trade terms. Generally, the terms and conditions of CIF require the seller to bear the cost of transporting the products to the port of discharge and the insurance cost, whereas those of CFR require the seller to bear only the cost of transporting the products to the port of discharge. In both cases, once the seller completes the export and customs clearance procedures and shipping at the port of export, the products are transferred to the buyer, and at that time, the risk burden is also transferred from the seller to the buyer (Exhibits Ko 104 and 105, Exhibits Otsu 45, 46, 102, 103, and 106).

c. Between the Defendant and CJ Indonesia, there was a commission contract under which CJ Indonesia was to pay the Defendant an amount equivalent to ●(omitted)●% or ●(omitted)●% of the sales amount of the portion sold by CJ Indonesia as a commission

(hereinafter the contract is referred to as the "Commission Contract" and the commission under this contract is referred to as the "Commission"), during the period of use of the Defendant's Production Processes. The total amount of the Commission paid from 2011 through 2017, which is the period of use of the Defendant's Production Processes, is ● (omitted)● yen (Exhibit Otsu 101).

d. As work related to transactions of the portion sold by CJ Indonesia, the Defendant at least engaged in delivery of samples and recovery of defective products of the MSG in question at various times, in addition to the forwarding of order forms mentioned in a. above, in Japan (Exhibit Otsu 101).

(D) The Defendant's accounting of the selling, general, and administrative expenses for the portion sold by the Defendant, etc.

a. During the period of use of the Defendant's Production Processes, the Defendant recorded, in accounting, expenses relating to the portion sold by the Defendant as well as expenses relating to the portion sold by CJ Indonesia under the expense items "freight expenses," "warehouse expenses," "personnel expenses," "service expenses," and "others," as selling, general, and administrative expenses concerning the sale of the MSG in question. When comparing the total amounts of the abovementioned selling, general, and administrative expenses that were recorded during the period of use of the Defendant's Production Processes, the ratio between the selling, general, and administrative expenses relating to the portion sold by the Defendant and those relating to the portion sold by CJ Indonesia was roughly 4:3 (Exhibit Otsu 101).

b. "Freight expenses" and "warehouse expenses"

In the Defendant's accounting, the standards based on which the "freight expenses" and the "warehouse expenses" concerning the sale of the MSG in question were to be distributed between the portion sold by the Defendant and the portion sold by CJ Indonesia were instructed by CJCJ.

Specifically, with regard to "freight expenses," all transportation expenses concerning the MSG in question were proportionally distributed according to the sales amount of the portion sold by the Defendant and the total amount of Commission until the middle of 2013. Subsequently, the transportation expenses were recorded as expenses for the portion sold by the Defendant, in principle, and only the expenses corresponding to indirect expenses for delivery of samples and recovery of defective products, etc. were proportionally distributed according to the sales amount of the portion sold by the Defendant and the total amount of Commission. Meanwhile, with regard to warehouse expenses, all warehouse expenses relating to the MSG in question were proportionally distributed according to the sales amount of the portion sold by the Defendant and the

total amount of Commission until 2016, and in 2017, all warehouse expenses were proportionally distributed according to the trading amount of the portion sold by the Defendant and the trading amount of the portion sold by CJ Indonesia (Exhibits Otsu 99 and 101).

c. "Personnel expenses," "service expenses," and "others"

In accounting, the Defendant recorded the selling, general, and administrative expenses concerning the sale of the MSG in question that are categorized under the expense items "personnel expenses," "service expenses," and "others" by the following procedure.

First, expenses such as "personnel expenses" that arose in the Defendant's organization overall were distributed among the respective departments according to the size of their trading amounts, etc. Then, of the expenses thus distributed to the "bio" department handling the MSG in question, expenses concerning the MSG in question were calculated according to the proportion of the sales of the MSG in question in the department's total sales. Further, those expenses concerning the MSG in question were distributed between the portion sold by the Defendant and the portion sold by CJ Indonesia according to their respective trading amounts. The standards for such distribution of expenses had been decided by agreement between the Defendant and CJCJ (Exhibits Otsu 99 and 101).

(E) Regarding the sales values and sales volumes of the Defendant's Products during the period of use of the Defendant's Production Processes

The sales values of the Defendant's Products relating to the portion sold by the Defendant and the portion sold by CJ Indonesia during the period of use of the Defendant's Production Processes (2011 through 2017) are as shown in 1. of Attachment 10 "List of Sales Values/Volumes," and the sales volumes thereof are as shown in 2. of that Attachment (an undisputed fact).

(F) Sales situation of the MSG in question to Japanese customers since 2018

Since 2018, when selling the MSG in question produced by CJ Indonesia to Japan, CJCJ has acted as the seller instead of CJ Indonesia, and CJCJ has directly sold the products to Japanese buyers, or has sold the products to the Defendant and then the Defendant sold them to Japanese buyers (Exhibits Otsu 99, 101, and 112).

B. Regarding working of the inventions in Japan with regard to the portion sold by the Defendant

While the MSG in question produced during the period of use of the Defendant's Production Processes represents products produced by any of the Defendant's Production Processes (the Defendant's Products) as mentioned in (1) above, the Defendant has

imported, transferred, and offered to transfer the MSG in question with regard to the portion sold by the Defendant during that period as mentioned in A. (B) above, and thus the Defendant is found to have worked Invention 1 or Invention 2 (Article 2, paragraph (3), item (iii) of the Patent Act).

C. Regarding working of the inventions in Japan with regard to the portion sold by CJ Indonesia

(A) Regarding assertion about an allegation or evidence presented after its time

The Defendant asserts that the allegation on the offer to transfer made by the Defendant, etc. with regard to the portion sold by CJ Indonesia should be dismissed without prejudice as an allegation or evidence presented after its time.

However, the allegation on the offer to transfer the MSG in question had been made from the stage of filing the complaint, as allegation on one of the acts of working the inventions conducted by the Defendant, and the Defendant's assertion that there is a portion sold by CJ Indonesia apart from the portion sold by the Defendant had not explicitly been made before the submission of Defendant's Brief 17 dated February 20, 2019 and the Defendant's Brief 18 dated April 18, 2019. Considering these facts, it cannot be said that the Plaintiff's act of alleging the offer to transfer as an act of working the inventions in relation to the portion sold by CJ Indonesia in Plaintiff's Brief 18 dated October 11, 2019 constitutes an act of additionally presenting an allegation after the time for doing so.

Consequently, the Defendant's abovementioned petition seeking dismissal of that allegation without prejudice as being an allegation or evidence presented after its time is to be dismissed without prejudice.

(B) Regarding import and transfer

a. According to the facts found in A. (C) above, the sales contracts for the portion sold by CJ Indonesia were concluded between CJ Indonesia and Japanese customers. Based on the CIF or CFR trade terms stated in the invoices upon transportation of the MSG in question to Japan (A. (C) b. above), it is reasonable to find that the transfer of the MSG in question to the buyers was conducted at the time of the shipping in Indonesia, and there is no sufficient evidence to find that, on the contrary, the MSG in question had been transferred to buyers after the discharge at the port of discharge or that the procedure for import to Japan had been conducted by CJ Indonesia, which is the seller.

If so, it is reasonable to find that the transfer of the MSG in question relating to the portion sold by CJ Indonesia had been conducted outside Japan, and also no fact can be found that the Defendant, etc. was importing them into Japan. It should be said that the import of the MSG in question relating to the portion sold by CJ Indonesia into Japan had

been conducted by the buyers to whom the MSG in question had been transferred.

b. Regarding the Plaintiff's allegations

The Plaintiff alleges that the portion sold by CJ Indonesia should actually be evaluated to have been imported into Japan and transferred in Japan by the Defendant, etc. However, even by taking into consideration the relationship between the Defendant and CJ Indonesia in the CJ Group mentioned in A. (A) above, the fact that order forms to be sent to CJ Indonesia were sometimes sent to the Defendant as mentioned in A. (C) a. above, the involvement of the Defendant in the portion sold by CJ Indonesia mentioned in A. (C) c. and d. above, and the processing in the Defendant's accounting mentioned in A. (D) above, they are not sufficient to overturn the determination mentioned in a. above that conclusion of the sales contract and the transfer of the MSG in question relating to the portion sold by CJ Indonesia are not found to have been conducted in Japan, and hence the Plaintiff's abovementioned allegation cannot be adopted.

In addition, the Plaintiff alleges as follows: [i] even if CJ Indonesia and Japanese clients concluded contracts and Japanese clients directly withdraw the MSG in question as cargo from the container yard, an act of bringing cargo into a bonded area should be construed to constitute an act of import with regard to goods that infringe patent rights, so CJ Indonesia's act constitutes an import; [ii] the act of transfer to Japanese clients by a shipping company, to which transportation has been entrusted by CJ Indonesia and which is deemed to act at the instruction of CJ Indonesia, is conducted in Japan, and therefore there was transfer in Japan by the Defendant, etc. However, given that the transfer of the MSG in question to the buyers is found to have been conducted at the time of the shipping in Indonesia, and that the MSG in question is not found to have been transferred to buyers after the discharge at the port of discharge as mentioned in a. above, with regard to the Plaintiff's allegation [i] above, it cannot be said that there was an act of import by the Defendant or CJ Indonesia, irrespective of whether or not an act of bringing cargo into a bonded area constitutes the act of import under Article 2, paragraph (3), item (iii) of the Patent Act, and for the same reason, the Plaintiff's allegation [ii] also cannot be adopted.

(C) Regarding the offer to transfer

a. As mentioned in (B) above, the transfer of the MSG in question itself is not found to have been conducted in Japan with regard to the portion sold by CJ Indonesia. However, it is reasonable to find that the Defendant was conducting sales activities relating to the portion sold by CJ Indonesia, jointly with CJ Indonesia, with regard to the portion sold by CJ Indonesia during the period of use of the Defendant's Production Processes, and the Defendant is found to have made an offer to transfer, in light of the following: the relationship between the Defendant and CJ Indonesia in the CJ Group mentioned in A.

(A) above; the fact that order forms for the portion sold by CJ Indonesia were sometimes submitted to the Defendant and were sent to CJ Indonesia via the Defendant as mentioned in A. (C) a. above; the fact that a Commission Contract was concluded between the Defendant and CJ Indonesia to the effect that CJ Indonesia would pay a part of the sales amount of the portion sold by CJ Indonesia to the Defendant as mentioned in A. (C) c. above; the fact that the Defendant engaged in delivery of samples and recovery of defective products of the MSG in question in Japan with regard to the portion sold by CJ Indonesia as mentioned in A. (C) d. above; and the fact that expenses relating to the portion sold by CJ Indonesia were recorded in the Defendant's accounting based on instructions by or based on an agreement with CJCJ as mentioned in A. (D) above.

In addition, since the MSG in question produced during the period of use of the Defendant's Production Processes represents products produced by any of the Defendant's Production Processes (the Defendant's Products) as mentioned in B. above, the Defendant's offer to transfer the MSG in question during that period constitutes the working of Invention 1 or Invention 2 (Article 2, paragraph (3), item (iii) of the Patent Act) by the Defendant.

b. Regarding the Defendant's allegations

(a) The Defendant alleges that an "offer to transfer" is an act to be conducted by the seller, which is the future transferor, and that an offer to transfer is not established in a case where the person that conducts the act of offering, by means such as advertising, differs from the person who makes the transfer, and therefore even if the Defendant, which is not the seller, becomes involved in some way with regard to the portion sold by CJ Indonesia, that act does not constitute an offer to transfer.

However, in light of the purport that an offer to transfer is stipulated as an act of working that is separate from a transfer, even if an act of offering to transfer is not conducted by the seller, which is the transferor, it should be considered that the act of offering can be construed to be an offer to transfer as long as there are circumstances such as that the act has been conducted by a person that has a certain relationship with the seller. Meanwhile, according to the results of the examination made in a. above, it can be said that CJ Indonesia and the Defendant not only belong to the same corporate group, but also had a close relationship with each other, in areas such as concluding a Commission Contract and sharing profits with regard to the portion sold by CJ Indonesia. Therefore, the fact that the party to the sales contract for the portion sold by CJ Indonesia was CJ Indonesia and not the Defendant does not preclude the finding that the Defendant's involvement mentioned in a. above constitutes an offer to transfer the MSG in question.

(b) The Defendant also alleges that, as a "transfer" under the Patent Act means a transfer

in Japan, and an "offer to transfer," which is an act to prepare for a transfer, also means an offer to make a transfer in Japan, the Defendant's act with regard to the portion sold by CJ Indonesia does not constitute an offer to transfer.

Indeed, although the transfer of the MSG in question to the buyers with regard to the portion sold by CJ Indonesia is found to have been conducted outside Japan as mentioned in (B) a. above, the portion sold by CJ Indonesia was all sold to buyers in Japan, and even if the transfer of the MSG in question itself was conducted at the time of the shipping, the MSG in question was scheduled to be subsequently imported into Japan by the buyers' side. In light of the purport that an offer to transfer is stipulated as a separate act of working from a transfer, regarding products like the MSG in question relating to the portion sold by CJ Indonesia, which are sold to buyers in Japan and imported into Japan as a result of sales activities conducted in Japan, it is not reasonable if the conclusion as to whether or not the sales activities constitute an offer to transfer made in Japan would differ solely based on whether the transfer to the buyers is conducted outside Japan or inside Japan. As mentioned in a. above, it is reasonable to construe that the act of the Defendant and CJ Indonesia to jointly conduct sales activities relating to the portion sold by CJ Indonesia, in Japan, constitutes an "offer to transfer" by the Defendant, the Defendant's allegation relating to this point cannot be adopted.

D. Regarding joint tort by the Defendant and CJ Indonesia

(A) As mentioned in B. and C. above, during the period of use of the Defendant's Production Processes, the Defendant worked the inventions relating to Patent Right 1 or Patent Right 2 by way of importing, transferring, and offering to transfer the MSG in question with regard to the portion sold by the Defendant, and by way of offering to transfer the MSG in question with regard to the portion sold by CJ Indonesia.

(B) As mentioned in A. (A) above, the Defendant and CJ Indonesia belong to the CJ Group which is centered around CJCJ, the wholly owning parent company of CJ Indonesia, and in that group, the group companies shared roles in producing and selling CJ-brand and other products in countries around the world.

With regard to sales of the MSG in question during the period of use of the Defendant's Production Processes, two modes of contract coexisted: one for the portion sold by the Defendant and the other for the portion sold by CJ Indonesia. Although they differed on whether or not the Defendant was included in the sales channel when products were sold to Japanese customers, they both sold the same product, the MSG in question produced by CJ Indonesia, to the Japanese market, and as examined in C. (C) above, the Defendant, which is not party to the sales contract, is found to have conducted sales activities also with regard to the portion sold by CJ Indonesia, while receiving a share of

the profits therefrom, and to have engaged in delivery of samples and recovery of defective products with regard to that portion, in the same manner as those for the portion sold by the Defendant. In addition, as mentioned in A. (D) above, it is found that, in the Defendant's accounting, overall amounts of transportation expenses, warehouse expenses, etc. concerning sales of the MSG in question were distributed between the portion sold by the Defendant and the portion sold by CJ Indonesia based on instructions by CJCJ, the parent company of CJ Indonesia.

In light of these facts, it is reasonable to find that the Defendant and CJ Indonesia are not merely in a relationship of a supplier/customer of the MSG in question with regard to the portion traded by the Defendant, but are in an integral relationship with each other in selling the MSG in question produced by CJ Indonesia targeting the Japanese market, regardless of the selling modes, i.e. that for the portion sold by the Defendant and that for the portion sold by CJ Indonesia, and that they jointly conduct the respective acts of working the inventions mentioned in (A) above with regard to the portion sold by the Defendant and the portion sold by CJ Indonesia, and it is reasonable to find that joint tort will be established for the Defendant, etc. in regard to these acts.

(3) Regarding the value of damages under Article 102, paragraph (2) of the Patent Act for the entire portion sold by the Defendant, etc. (Attachment 11-1 "Allegation on the Value of Damages 1")

As mentioned in No. 3, 15. [Plaintiff's allegations] (3) above, the Plaintiff has made allegations on the value of damages sustained by the Plaintiff, based on the four calculation methods described in the [Plaintiff's allegations] columns in Attachment 11-1 "Allegation on the Value of Damages 1" through Attachment 11-4 "Allegation on the Value of Damages 4." First, the allegation on the value of damages under Article 102, paragraph (2) of the Patent Act for the entire portion sold by the Defendant, etc., relating to Attachment 11-1"Allegation on the Value of Damages 1," will be examined.

A. Meaning of "profit" under Article 102, paragraph (2) of the Patent Act

The amount of profit which the infringer has made from the infringement prescribed in Article 102, paragraph (2) of the Patent Act is the amount of marginal profit obtained by deducting expenses that became additionally required in direct association with the sale of the infringing goods by the infringer as a result of selling the infringing goods from the amount of sales of infringing goods by the infringer.

B. Method of calculation of the profit under Article 102, paragraph (2) of the Patent Act made through joint tort by the Defendant, etc.

(A) As mentioned in (2) above, the inventions relating to Patent Right 1 or Patent Right 2 have been worked by way of importing, transferring, and offering to transfer the MSG

in question with regard to the portion sold by the Defendant, and by way of offering to transfer the MSG in question with regard to the portion sold by CJ Indonesia, and joint tort by the Defendant and CJ Indonesia is found to be established for these acts. Accordingly, in presuming the value of damages under Article 102, paragraph (2) of the Patent Act as well, it should be said that the total amount of the marginal profits of the Defendant, etc. calculated by the calculation methods mentioned in A. above would be presumed to be the value of damages sustained by the Plaintiff with regard to the abovementioned infringing acts relating to the portion sold by the Defendant, etc.

Also considering that the Defendant and CJ Indonesia were in an integral relationship with each other in selling the MSG in question produced by CJ Indonesia targeting the Japanese market, regardless of the selling modes, i.e. that for the portion sold by the Defendant and that for the portion sold by CJ Indonesia, as mentioned in (2) above, it is reasonable to calculate the total amount of the marginal profits made by the Defendant, etc. through the respective infringing acts relating to the portion sold by the Defendant, etc. by deducting overall expenses that became additionally required by Defendant, etc. in direct association with the sale of the relevant portion from the overall sales amount of the portion sold by the Defendant, etc.

(B) Regarding this point, the Defendant alleges that the value of damages from the "offer to transfer," which is an act of working the inventions in relation to the portion sold by CJ Indonesia, should not be calculated based on the sales amount of the portion sold by CJ Indonesia, but instead should remain within the scope specific to the "offer to transfer."

However, because joint tort is established for the Defendant and CJ Indonesia with regard to the portion sold by CJ Indonesia as mentioned in (2) D. above., not only the profits made by the Defendant but also those made by CJ Indonesia would be considered in calculating the value of damages, and, as examined in (2) C. (C) b. (b) above, the portion sold by CJ Indonesia was all sold to buyers in Japan, and was scheduled to be subsequently imported into Japan after being transferred to the buyers. Therefore, it is reasonable to find that, even if the "transfer" itself was conducted outside Japan, the profits made by the Defendant, etc. calculated based on the sales amount of the portion sold by CJ Indonesia are the profits received by the Defendant, etc. from the "offer to transfer" made in Japan, in applying Article 102, paragraph (2) of the Patent Act, and the Defendant's abovementioned allegation cannot be adopted.

C. Sales amount of the portion sold by the Defendant, etc.

As mentioned in (2) A. (E) above, the sales values of the Defendant's Products relating to the portion sold by the Defendant, etc. during the period of use of the Defendant's Production Processes (2011 through 2017) are as shown in 1. of Attachment 10 "List of

Sales Values/Volumes."

D. Expenses borne by the Defendant, etc. to be deducted from the sales amount

(A) Sales volumes

As mentioned in (2) A. (E) above, the sales volumes of the Defendant's Products relating to the portion sold by the Defendant, etc. during the period of use of the Defendant's Production Processes are as shown in 2. of Attachment 10 "List of Sales Values/Volumes."

(B) Regarding the production expenses for the MSG in question

a. Eligibility of expenses

As the MSG in question relating to the portion sold by the Defendant, etc. was all produced by CJ Indonesia, it should be said that its production expenses are eligible as expenses to be deducted in calculating the marginal profits of the Defendant, etc. as expenses that became additionally required in direct association with the sale of the portion sold by the Defendant, etc.

b. Production expenses for the MSG in question per ton

According to evidence (Exhibit Otsu 107) and the entire import of oral arguments, it is reasonable to find that, as the production expenses for the MSG in question during the period of use of the Defendant's Production Processes, the annual total amounts of fermentation material expenses, purification material expenses, utility expenses, and packaging expenses per ton of the MSG in question were the amounts stated in [Plaintiff's allegations] 2. (2) B. (A) of Attachment 11-1 "Allegation on the Value of Damages 1" (Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Overall Portion Sold by the Defendant, etc.) (on a U.S. dollar basis; values for 2015 through 2017 are the same as the value for 2014).

Meanwhile, the abovementioned evidence (Exhibit Otsu 107) disclosed by the Defendant also states expenses titled "fixed expenses (labor, depreciation, and others)" as expenses relating to production of the MSG in question, but as they cannot be considered as expenses in direct association with the sale of the MSG in question in light of their nature, they should not be considered in calculating the marginal profits.

c. Exchange rates

According to evidence (Exhibit Ko 106), it is found that the exchange rates of U.S. dollars to yen during the period of use of the Defendant's Production Processes were as shown in [Plaintiff's allegations] 2. (2) B. (B) of Attachment 11-1 "Allegation on the Value of Damages 1" (Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Overall Portion Sold by the Defendant, etc.).

d. Amounts of expenses to be deducted

Then, when the sales volumes of the portion sold by the Defendant, etc. mentioned in (A) above are multiplied by the production expenses per ton mentioned in b. above and the exchange rates mentioned in c. above, the production expenses for the MSG in question during the period of use of the Defendant's Production Processes in relation to the portion sold by the Defendant, etc., which should be deducted when calculating the marginal profits, would be as shown in 3. (i)-3 of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc."

(C) Regarding expenses for transportation of the MSG in question to Japan

a. Eligibility of expenses

As the MSG in question relating to the portion sold by the Defendant, etc. was all produced at CJ Indonesia's factory in Indonesia and was sent to Japan, and, according to the statements in the invoices mentioned in (2) A. (C) b. above, CIF or CFR trade terms under which CJ Indonesia, the seller, was to bear the cost of transporting the products to the port of discharge with regard to the portion sold by CJ Indonesia are considered to have been adopted, it should be said that the expenses for transporting the MSG in question to Japan in relation to the portion sold by the Defendant, etc. are eligible as expenses to be deducted in calculating the marginal profits of the Defendant, etc. as expenses that became additionally required in direct association with the sale of the portion sold by the Defendant, etc.

b. Per-ton expenses for transporting the MSG in question to Japan

According to evidence (Exhibit Otsu 113) and the entire import of oral arguments, it is reasonable to find that the annual average amounts of the per-ton expenses for transporting the MSG in question to Japan relating to the portion sold by the Defendant, etc. during the period of use of the Defendant's Production Processes were the amounts stated in [Plaintiff's allegations] 2. (2) C. (A) of Attachment 11-1 "Allegation on the Value of Damages 1" (Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Overall Portion Sold by the Defendant, etc.).

c. Amounts of expenses to be deducted

When the sales volumes of the portion sold by the Defendant, etc. mentioned in (A) above are multiplied by the per-ton expenses for transportation mentioned in b. above and the exchange rates mentioned in (B) c. above, the expenses for transporting the MSG in question to Japan during the period of use of the Defendant's Production Processes in relation to the portion sold by the Defendant, etc., which should be deducted when calculating the marginal profits, would be as shown in 3. (ii) of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent

Act for the Portion Sold by the Defendant, etc."

(D) Regarding domestic freight expenses and other selling, general, and administrative expenses

As mentioned in (2) A. (D) above, the Defendant had recorded expenses under the expense items "freight expenses," "warehouse expenses," "personnel expenses," "service expenses," and "others" as selling, general, and administrative expenses relating to the portion sold by the Defendant, etc. The eligibility of these expense items as expenses and the amounts of expenses to be deducted will be examined.

a. Regarding "freight expenses" and "warehouse expenses"

According to evidence (Exhibits Otsu 99 and 101) and the entire import of oral arguments, it is found that the amounts of expenses recorded as "freight expenses" and "warehouse expenses" for the MSG in question relating to the portion sold by the Defendant, etc. during the period of use of the Defendant's Production Processes were the amounts respectively shown for the portion sold by the Defendant and the portion sold by CJ Indonesia in 3. (iii) of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc." (the values for the portion sold by the Defendant are the same as those stated in [Plaintiff's allegations] 2. (2) D. of Attachment 11-1 "Allegation on the Value of Damages 1" (Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Overall Portion Sold by the Defendant, etc.)). Meanwhile, the respective warehouse expenses for 2017 found above are those after correcting the distribution of the respective expenses stated in Reference Materials 3 attached to Exhibit Otsu 101 between the portion sold by the Defendant and the portion sold by CJ Indonesia, without changing the total amounts for the two portions, according to the allegation made in Defendant's Brief 19.

In addition, according to evidence (Exhibits Otsu 99 and 101) and the entire import of oral arguments, these expenses recorded as "freight expenses" and "warehouse expenses" are found to be those wherein the total amount of the expenses actually required by the Defendant for transporting and storing the MSG in question relating to the portion sold by the Defendant, etc. was distributed between the portion sold by the Defendant and the portion sold by CJ Indonesia in accounting, as mentioned in (2) A. (D) b. above. Therefore, it should be said that the amount obtained by adding the amount of expenses distributed to the portion sold by the Defendant, which the Plaintiff recognized as expenses, and the amount of expenses distributed to the portion sold by CJ Indonesia is eligible as expenses to be deducted in calculating the marginal profits of the Defendant, etc. as expenses that became additionally required in direct association with the sale of the portion sold by the Defendant, etc.

b. Regarding other selling, general, and administrative expenses

As for the expense items "personnel expenses," "service expenses," and "others," which the Defendant recorded as selling, general, and administrative expenses relating to the sale of the MSG in question, the relevant portion of the expenses, such as "personnel expenses," that arose in the Defendant's organization overall was distributed to the Defendant's "bio" department in accounting, and a part thereof was further distributed between the portion sold by the Defendant and the portion sold by CJ Indonesia as mentioned in (2) A. (D) c. above. As they cannot be considered as expenses in direct association with the sale of the MSG in question in light of their nature, they are not eligible as expenses to be deducted in calculating the marginal profits of the Defendant, etc.

The Defendant alleges that, while "personnel expenses" include the personnel expenses of operation departments and those of indirect sections, at least the personnel expenses of operation departments depend on the workload of sales operations, so they should be deducted as expenses that became additionally required in direct association with the sale of the MSG in question. However, given the method by which the amount of the "personnel expenses" recorded for the portion sold by the Defendant, etc. has been decided, the personnel expenses of operation departments, which constitute a part thereof, also cannot be considered as expenses in direct association with the sale of the portion sold by the Defendant, etc. in light of their nature, and hence the Defendant's allegation cannot be adopted.

(E) Regarding purchasing expenses

The Defendant alleges that, when considering the profits for the portion sold by the Defendant, the expenses required for their purchase should be taken into consideration.

As mentioned in B. (B) above, the Defendant, etc. are in an integral relationship with regard to the portion sold by the Defendant, etc., and joint tort by the Defendant, etc. is established for the portion sold by the Defendant, etc. Thus, the total amount of profits made by the Defendant and CJ Indonesia should also be calculated when considering the profits for the portion sold by the Defendant. Consequently, is should be said that the purchasing expenses that arose when the Defendant purchased the MSG in question from CJ Indonesia with regard to the portion sold by the Defendant are not eligible as expenses to be deducted from the sales amount of the portion sold by the Defendant when calculating the total amount of profits made by the Defendant, etc. with regard to the portion sold by the Defendant.

(F) Summary

According to the above, expenses to be deducted from the sales amount of the portion

sold by the Defendant, etc. when calculating the total amount of profits made by the Defendant, etc. are the production expenses for the MSG in question mentioned in (B) above, the expenses for transportation of the MSG in question to Japan mentioned in (C) above, and the domestic freight expenses and warehouse expenses mentioned in (D) a. above (the total amounts of the respective expenses to be deducted are as shown in 4. of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc.").

E. Total amount of marginal profits made by the Defendant, etc.

According to the above, the total amount of the marginal profits made by the Defendant, etc. through the respective infringing acts relating to the portion sold by the Defendant, etc. during the period of use of the Defendant's Production Processes is the amount obtained by deducting, from the sales amount of the portion sold by the Defendant, etc. mentioned in C. above, the expenses to be deducted mentioned in D. (F) above, and as shown in 4. of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc.," the total amount throughout the period comes to 3,857,313,065 yen.

F. Regarding grounds for overturning the presumption

(A) Regarding the sale of competitive products (reducing the amount according to market share)

a. According to evidence (Exhibits Otsu 101 and 117) and the entire import of oral arguments, the volumes of monosodium glutamate imported from the respective countries into Japan during the period of use of the Defendant's Production Processes are as shown in column (i) through column (x) of Attachment 16 "Situation of Import of Monosodium Glutamate During the Period of Use of the Defendant's Production Processes." Of these, the entire volumes of the imports from (i) Thailand and (ii) Brazil are found to be those imported and sold in Japan by the Plaintiff, and the imports from (iii) Indonesia are found to include not only the portion sold by the Defendant, etc. ((iii) A.), but also those imported by the Plaintiff. Meanwhile, in calculating the volumes for 2017, the volumes stated in Reference Materials 7 attached to Exhibit Otsu 101 were adopted instead of those in Reference Materials 6 attached to the same as reference materials similar to the reference materials for 2011 through 2016 (Exhibit Otsu 117).

b. The MSG in question is monosodium glutamate, and as mentioned in a. above, the monosodium glutamate mentioned in a. above, which was imported during the period of use of the Defendant's Production Processes, is all found to constitute products that are in competitive relationship with the MSG in question relating to the portion sold by the Defendant, etc. as equivalents thereto in the Japanese market.

In this manner, the fact that the abovementioned competitive products existed apart from the portion sold by the Defendant, etc. and the portion imported and sold by the Plaintiff should be considered as circumstances for overturning the presumption under Article 102, paragraph (2) of the Patent Act, with regard to a part of the total amount of marginal profits made by the Defendant, etc. mentioned in E. above.

c. The percentage to be overturned due to the abovementioned circumstances will be examined below.

With regard to the volume ((iii) B) other than the portion sold by the Defendant, etc. ((iii) A) out of the volume imported from (iii) Indonesia mentioned in a. above, the Defendant alleges that half of such other portion is presumed to have been imported by business operators other than the Plaintiff. However, there is no evidence supporting the volume imported from Indonesia by business operators other than the Plaintiff.

Considering the Plaintiff's allegation that most of that volume ((iii) B) was imported by the Plaintiff, the portion imported and sold by the Plaintiff ((xi)) was calculated by assuming that volume to have been imported by the Plaintiff. Then, the percentage of the portion imported and sold by the Plaintiff ((xi)) in the volume obtained by deducting the portion sold by the Defendant, etc. ((iii) A) from the total import volume ((x)) was calculated. As a result, the annual percentages during the period of use of the Defendant's Production Processes came to ●(omitted)●% to ●(omitted)●% as stated in the Attachment, and the average of the annual percentages came to 44.7%.

With regard to the reference materials of trade statics by the Ministry of Finance submitted by the Defendant (Reference Materials 3 through 10 of Exhibit Otsu 117), the Plaintiff also alleges that these reference materials do not include the volume of imports that are imported as glutamic acid and processed into monosodium glutamate in Japan, and that most of those were imports by the Plaintiff. However, the Plaintiff has not submitted any specific reference material to support this allegation, and evidence (Reference Materials 7 attached to Exhibit Otsu 101) suggests that the import volume of such glutamic acid was small compared to the import volume of monosodium glutamate.

Then, even by considering the Plaintiff's allegation, it is reasonable to find that, even if the portion sold by the Defendant, etc. was not sold during the period of use of the Defendant's Production Processes, at least about 50% of the demand that had been applicable to the MSG in question relating to the portion sold by the Defendant, etc. would have been applicable to the monosodium glutamate imported and sold by business operators other than the Plaintiff, and accordingly, it is reasonable to find that the presumption is to be overturned by 50%.

(B) Regarding the degree of contribution to infringement of the Patent Rights

The Defendant alleges that the inventions claimed in the Patents only contribute to a limited part of the processes for producing glutamic acid comprising complicated and diverse steps and the degree of their respective contribution to production of the MSG in question is low, and that therefore, in calculating damages under Article 102, paragraph (2) of the Patent Act, the degree of contribution of the Patents should be considered or that the overturning of the presumption according to the degree of contribution should be allowed.

However, as Invention 1-1 (Corrected Invention 1-1), Invention 1-2 (Corrected Invention 1-2), and Invention 1-4 (Corrected Invention 1-3), as well as Invention 2-5 (Corrected Invention 2-5), which are found to have been worked during the period of use of the Defendant's Production Processes, are all inventions relating to process for producing glutamic acid (amino acid) by using strains having specific characteristics, if the working of the abovementioned patented inventions is found as a result of the sale, etc. of the MSG in question produced by using those strains, it should be said that the patented inventions have all been worked for the entirety of the MSG in question sold.

Even if production of glutamic acid by the fermentation method comprises the fermentation phase and the purification phase, and further, multiple reactions occur in the strains in the fermentation phase, and the abovementioned patented inventions only relate to a specific phase or reaction in that process, the degree of contribution of the abovementioned patented inventions to production of the MSG in question cannot be found to be low based on such reason. In addition, no other circumstances are found, in the evidence of the present case, due to which the presumption under Article 102, paragraph (2) of the Patent Act should be overturned with regard to the claims based on the Patent Rights based on the degree of contribution by the abovementioned patented inventions. Therefore, the Defendant's abovementioned allegation cannot be adopted.

G. Summary on the value of damages under Article 102, paragraph (2) of the Patent Act for the overall portion sold by the Defendant, etc.

(A) If the grounds for overturning the presumption mentioned in F. above are taken into consideration with regard to the total amount of the marginal profits made by the Defendant, etc. mentioned in E. above, the value of damages sustained by the Plaintiff under Article 102, paragraph (2) of the Patent Act comes to a total of 1,928,656,532 yen.

As this amount exceeds the value of damages of 900 million yen sought by the Plaintiff as the partial claim for the value of damages calculated under Article 102, paragraph (2) or (3) of the Patent Act, there is no need to determine the appropriateness of overlapped application of Article 102, paragraph (3) of the Patent Act for the overturned part mentioned in F. above or the allegations stated in the [Plaintiff's

allegations] columns in Attachment 11-2 "Allegation on the Value of Damages 2" through Attachment 11-4 "Allegation on the Value of Damages 4."

(B) When the period for which exercise of rights based on the Patent Rights mentioned in (1) above is taken into consideration with regard to the value of damages mentioned in (A) above, and the correspondence between the damages that occurred in the respective periods and the Patent Rights is examined, the following can be derived (the respective calculation results are as shown in 5. of Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc.").

a. January 2011 through August 22, 2013

A period during which Patent Right 1 was worked; a total of 466,184,463 yen

b. August 23, 2013 (the date of registration of establishment of Patent Right 2) through June 2016

A period during which Patent Rights 1 and 2 were worked; a total of 897,628,767 yen

c. July 2016 through December 2017

A period during which Patent Right 2 was worked; a total of 564,843,302 yen

(C) With regard to the relationship between the claims based on Patent Right 1 and Patent Right 2, the Plaintiff has selectively made claims based on each patent right within the scope of 900 million yen, which is the amount of the partial claim. According to (B) above, the values of damages caused by working Patent Right 1 and Patent Right 2 are respectively found to exceed 450 million yen, which is half the amount of 900 million yen. Therefore, it is reasonable to distribute the value of damages mentioned in (A) above by the ratio of 1:1, which is the ratio of the amounts of partial claims, and to affirm 450 million yen for the claim based on Patent Right 1 and 450 million yen for the claim based on Patent Right 2.

(4) Regarding lawyer fees and patent attorney fees

Considering the complexity of the present case, the amounts of partial claims in the claims for compensation for damages in the present case, and the amounts affirmed mentioned in (3) above, among other matters, it is reasonable to find that lawyer fees and patent attorney fees that have causal links with the joint tort of infringement of the Patent Rights by the Defendant, etc. are 90 million yen (45 million yen for infringement of Patent Right 1 and 45 million yen for infringement of Patent Right 2).

(5) Summary on the claim for compensation for damages

According to the above, without having to make determinations on other points, the Plaintiff's claims for compensation for damages against the Defendant based on tort of infringement of the Patent Rights (a partial claim for the total value of damages of 990

million yen, and a claim for delay damages accrued thereon at the rate of 5% per annum as prescribed in the Civil Code for the period from August 10, 2016, the day following the date of service of the complaint against this value) are all found to be well-grounded.
9. Regarding Issue 9 (whether or not the claim seeking injunction and the claim seeking disposal are appropriate)
(1) Regarding the claims based on Patent Right 1

As mentioned in No. 2, 2. (3) above, the term of Patent Right 1 expired on September 22, 2019. Therefore, the claim seeking injunction and the claim seeking disposal based on Patent Right 1 are both groundless.
(2) Regarding the claims based on Patent Right 2
A. With regard to the period of use of the Defendant's Production Processes, as examined in 1. (4) above, it is found that the Defendant's Production Process 1 was used for the periods of use of Strains (ii) and (x) stated in Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question," and other periods of use are as shown in the "Plaintiff's alternative claims" column of that Attachment. After the use of Strain (xiii) ended in December 2017, the Defendant's Production Processes that are included in the technical scope of Invention 2-5 (Corrected Invention 2-5) are not found to have been used for producing the MSG in question.

However, given that the strains used for producing the MSG in question were frequently changed during the period of use of the Defendant's Production Processes as mentioned above, it is considered to have been easy for CJ Indonesia to change the strain again and produce the MSG in question by the Defendant's Production Processes. Consequently, even at this point of time, there is found to be a need for the Plaintiff to suspend transfer, import, or offer to transfer (including display for the purpose of transfer) of the MSG in question produced by the Defendant's Production Processes and seek their disposal under Article 100, paragraph (1) and paragraph (2) of the Patent Act based on Patent Right 2.
B. Products subject to injunction, etc.

As mentioned in 3. above, the strains used in Defendant's Production Processes 1 through 3 are all found to fall within the technical scope of Invention 2-5 as *Corynebacterium glutamicum* into which a yggB gene, wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 has been substituted with threonine, has been introduced.

In addition, as mentioned in 4. above, the strains used in Defendant's Production Process 4 (Strains (xii) and (xiii)) are both found to fall within the technical scope of Invention 2-5 as *Corynebacterium glutamicum* into which a yggB gene, wherein alanine

at position 98 of the amino acid sequence encoded by a yggB gene derived from the wild-type *Corynebacterium callunae* DSM20147 strain has been substituted with threonine and valine at position 241 of the sequence has been substituted with isoleucine, has been introduced.

Accordingly, it should be said that the products for which the injunction and disposal mentioned in A. above should be sought based on Patent Right 2 would be specified as shown in 1. and 2. of Attachment 17 "List of Products Subject to Injunction" (meanwhile, the amino acid sequence indicated in the sequence listing attached to that Attachment is the amino acid sequence of SEQ ID NO: 6 in Description 2).

10. Conclusion

According to above, the Plaintiff's claim seeking injunction and disposal based on the Patent Rights filed against the Defendant are well-grounded to the extent of seeking injunction and disposal with regard to the act of transferring, importing, and offering to transfer the monosodium glutamate mentioned in 1. and 2. of Attachment 17 "List of Products Subject to Injunction" based on Patent Right 2, and the Plaintiff's claims for compensation for damages caused by infringement of the Patent Rights are all well-grounded to the extent of the Plaintiff filing partial claims in the present case, whereas the Plaintiff's other claims are all groundless; therefore, the judgment is rendered as indicated in the main text. As it is not reasonable to attach a declaration of provisional execution to paragraph (2) of the main text, the declaration shall not be attached to that paragraph.

Tokyo District Court, 29th Civil Division

Judge: YANO Norio

Presiding judge YAMADA Maki and judge NISHIYAMA Yoshiki were unable to sign and seal due to a transfer of position.

Judge: YANO Norio

List of Attachments

(omitted)

Attachment 1 "List of Parties"
Plaintiff: Ajinomoto Co., Inc.

(omitted)

Defendant: CJ Japan Corp.

(omitted)

Attachment 2 "List of the Defendant's Products"

Monosodium glutamate with the product name "MI-POONG"
which satisfies the following requirements:

1. Defendant's Product 1

One produced by using *Corynebacterium glutamicum* wherein a TTGTCA sequence has been introduced in the -35 region and a TATAAT sequence has been introduced in the -10 region of the promoter sequence of the glutamate dehydrogenase (GDH) gene (the GDH gene), a TATAAT sequence has been introduced in the -10 region of the promoter sequence of the citrate synthetase (CS) gene (the gltA gene), and alanine at position 100 of the amino acid sequence encoded by a yggB gene has been substituted with threonine

2. Defendant's Product 2

One produced by using *Corynebacterium glutamicum* wherein alanine at position 100 of the amino acid sequence encoded by a yggB gene has been substituted with threonine

3. Defendant's Product 3

One produced by using *Corynebacterium glutamicum* wherein a TATAAT sequence has been introduced in the -10 region of the promoter sequence of the citrate synthetase (CS) gene (the gltA gene), and alanine at position 100 of the amino acid sequence encoded by a yggB gene has been substituted with threonine

4. Defendant's Product 4

One produced by using *Corynebacterium glutamicum* into which a yggB gene of *Corynebacterium callunae*, wherein alanine at position 98 of the amino acid sequence encoded by a yggB gene has been substituted with threonine, has been introduced

End

Attachment 3 "List of the Defendant's Production Processes"

1. A process of producing monosodium glutamate which is the product mentioned in 1. of Attachment 2 "List of the Defendant's Products," comprising the following steps: *Corynebacterium glutamicum*, which is a coryneform bacterium having the characteristics mentioned in 1. of Attachment 2 "List of the Defendant's Products" in the gene on the chromosome, is placed in a fermentation vessel, or, in a medium containing ingredients, such as the carbon source, and are propagated; L-glutamic acid is produced by the fermentation method whereby the carbon source is converted into L-glutamic acid through metabolic reactions within the bacterial cells; after the end of this fermentation process, the supernatant liquid containing the fermentation bacterium (bacterial cells) and L-glutamic acid are separated and collected; and the collected glutamic acid is neutralized by caustic soda

2. A process of producing monosodium glutamate which is the product mentioned in 2. of Attachment 2 "List of the Defendant's Products" by using the same process as in 1. above, except for using *Corynebacterium glutamicum* which is a coryneform bacterium having the characteristics mentioned in 2. of Attachment 2 "List of the Defendant's Products" in the gene on the chromosome

3. A process of producing monosodium glutamate which is the product mentioned in 3. of Attachment 2 "List of the Defendant's Products" by using the same process as in 1. above, except for using *Corynebacterium glutamicum* which is a coryneform bacterium having the characteristics mentioned in 3. of Attachment 2 "List of the Defendant's Products" in the gene on the chromosome

4. A process of producing monosodium glutamate which is the product mentioned in 4. of Attachment 2 "List of the Defendant's Products" by using the same process as in 1. above, except for using *Corynebacterium glutamicum* which is a coryneform bacterium having the characteristics mentioned in 4. of Attachment 2 "List of the Defendant's Products" in the gene on the chromosome

End

Attachment 4-1 "Claims (Patent 1)"

[Claim 1] (Invention 1-1)
A process for producing L-glutamic acid or L-arginine by the fermentation, which comprises the steps of culturing a bacterium in a medium to form and also accumulate L-glutamic acid or L-arginine in the medium, and collecting L-glutamic acid or L-arginine from the medium, wherein the bacterium is a coryneform bacterium in which at least one DNA sequence selected from the group consisting of TTGTCA, TTGACA, TTGCTA, and TTGCCA is introduced into -35 region of the promoter sequence of a gene selected from the group consisting of glutamate dehydrogenase (GDH), citrate synthase (CS), isocitrate dehydrogenase (ICDH), pyruvate dehydrogenase (PDH), and argininosuccinate synthase on a chromosome of the coryneform bacterium and/or TATAAT sequence or TATAAC sequence is introduced into -10 region of that promoter sequence.
[Claim 2] (Invention 1-2)
A process according to Claim 1, wherein a promoter of glutamate dehydrogenase (GDH)-producing gene has at least one DNA sequence selected from the group consisting of TTGTCA, TTGACA, and TTGCCA in -35 region and/or TATAAT sequence in -10 region.
[Claim 3] (Invention 1-3)
A process according to Claim 2, wherein a promoter of GDH has TTGACA sequence or TTGCCA sequence in -35 region and/or TATAAT in -10 region.
[Claim 4] (Invention 1-4)
A process according to Claim 1, wherein a promoter of CS has TTGACA sequence in -35 region and/or TATAAT sequence or TATAAC sequence in -10 region.

End

Attachment 4-2 "Corrected Claims (Patent 1)"

[Claim 1] (Corrected Invention 1-1)
A process for producing L-glutamic acid by the fermentation, which comprises the steps of culturing a bacterium in a medium to form and also accumulate L-glutamic acid in the medium, and collecting L-glutamic acid from the medium, wherein the bacterium is a coryneform bacterium in which TTGTCA sequence is introduced into -35 region and TATAAT sequence is introduced into -10 region of the promoter sequence of glutamate dehydrogenase (GDH) gene on a chromosome of the coryneform bacterium and/or TATAAT sequence is introduced into -10 region of the promoter sequence of citrate synthase (CS) gene on a chromosome of that bacterium.
[Claim 2] (Corrected Invention 1-2)
A process according to Claim 1, wherein the promoter of GDH gene has TTGTCA sequence in -35 region and TATAAT sequence in -10 region.
[Claim 3] (Deleted)
[Claim 4] (Corrected Invention 1-3)
A process according to Claim 1, wherein the promoter of CS gene has TATAAT sequence in -10 region or has TTGACA sequence in -35 region and TATAAT sequence in -10 region, and the promoter of GDH gene has TTGTCA sequence in -35 region and TATAAT sequence in -10 region.

End

Attachment 4-3 "Division into Constituent Features (Invention 1)"

Invention 1-1 (Claim 1)

1-B A process for producing L-glutamic acid or L-arginine by the fermentation, which comprises the steps of culturing a bacterium in a medium to form and also accumulate L-glutamic acid or L-arginine in the medium, and collecting L-glutamic acid or L-arginine from the medium,

1-A-3 wherein the bacterium is a coryneform bacterium in which

1-A-1 at least one DNA sequence selected from the group consisting of TTGTCA, TTGACA, TTGCTA, and TTGCCA is introduced into -35 region of the promoter sequence of a gene selected from the group consisting of glutamate dehydrogenase (GDH), citrate synthase (CS), isocitrate dehydrogenase (ICDH), pyruvate dehydrogenase (PDH), and argininosuccinate synthase on a chromosome of the coryneform bacterium and/or

1-A-2 TATAAT sequence or TATAAC sequence is introduced into -10 region of that promoter sequence.

Invention 1-2 (Claim 2)

1-D A process according to Claim 1,

1-C-1 wherein a promoter of glutamate dehydrogenase (GDH)-producing gene has at least one DNA sequence selected from the group consisting of TTGTCA, TTGACA, and TTGCCA in 35 region and/or

1-C-2 TATAAT sequence in -10 region.

Invention 1-3 (Claim 3)

1-F A process according to Claim 2,

1-E-1 wherein a promoter of GDH has TTGACA sequence or TTGCCA sequence in -35 region and/or

1-E-2 TATAAT in -10 region.

Invention 1-4 (Claim 4)

1-H A process according to Claim 1,

1-G-1 wherein a promoter of CS has TTGACA sequence in -35 region and/or

1-G-2 TATAAT sequence or TATAAC sequence in -10 region.

End

Attachment 4-4 "Division into Constituent Features (Corrected Invention 1)"

Corrected Invention 1-1 (Claim 1)

1-B' A process for producing L-glutamic acid by the fermentation, which comprises the steps of culturing a bacterium in a medium to form and also accumulate L-glutamic acid in the medium, and collecting L-glutamic acid from the medium,

1-A-3 wherein the bacterium is a coryneform bacterium in which

1-A'-1 TTGTCA sequence is introduced into -35 region and TATAAT sequence is introduced into -10 region of the promoter sequence of glutamate dehydrogenase (GDH) gene on a chromosome of the coryneform bacterium and/or

1-A'-2 TATAAT sequence is introduced into -10 region of the promoter sequence of citrate synthase (CS) gene on a chromosome of that bacterium.

Corrected Invention 1-2 (Claim 2)

1-D A process according to Claim 1,

1-C'-1 wherein the promoter of GDH gene has TTGTCA sequence in -35 region and

1-C'-2 TATAAT sequence in -10 region.

Corrected Invention 1-3 (Claim 4)

1-H A process according to Claim 1,

1-G' wherein the promoter of CS gene has TATAAT sequence in -10 region or has TTGACA sequence in -35 region and TATAAT sequence in -10 region, and

1-G" the promoter of GDH gene has TTGTCA sequence in -35 region and TATAAT sequence in -10 region.

Attachment 5-1 "Claims (Patent 2)"

[Claim 1] (Invention 2-1)
A coryneform bacterium having an L-glutamic acid-producing ability,
wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,
wherein any of the following mutations (i), (i'), (i''), or (ii) is introduced into the mutant-type yggB gene:
(i) deletion of a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;
(i') insertion of an insertion sequence or a transposon into a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;
(i'') a mutation in which proline in a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62 is substituted with another amino acid;
or
(ii) substitution, deletion, or insertion of one or several amino acids in a region selected from the group consisting of amino acid numbers 1 to 23, 86 to 108, and 110 to 132 of an amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.
[Claim 4] (Invention 2-2)
The coryneform bacterium according to Claim 1, wherein the mutation (ii) is a mutation in which alanine at position 100 and/or alanine at position 111 is substituted with another amino acid, in the amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.
[Claim 6] (Invention 2-3)
The coryneform bacterium according to any of Claims 1 to 5, wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (a) to (n):
(a) DNA encoding an amino acid sequence of SEQ ID NO: 8;
(b) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 8, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(c) DNA encoding an amino acid sequence of SEQ ID NO: 20;
(d) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 20, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(e) DNA encoding an amino acid sequence of SEQ ID NO: 22;
(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;
(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(i) DNA encoding an amino acid sequence of SEQ ID NO: 64;
(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(k) DNA encoding an amino acid sequence of SEQ ID NO: 70;
(l) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 70, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(m) DNA encoding an amino acid sequence of SEQ ID NO: 74; and
(n) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 74, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin.

[Claim 10] (Invention 2-4)
The coryneform bacterium according to any of Claims 1 to 9, wherein the coryneform bacterium belongs to the genus *Corynebacterium* or the genus *Brevibacterium*.
[Claim 11] (Invention 2-5)
A process for producing L-glutamic acid, comprising culturing a coryneform bacterium according to any of Claims 1 to 10 in a medium so as to cause production and accumulation of L-glutamic acid in the medium or the bacterium, and collecting the L-glutamic acid from the medium or the bacterium.

End

Attachment 5-2 "Corrected Claims (Patent 2)"

[Claim 1] (Corrected Invention 2-1)
A coryneform bacterium having an L-glutamic acid-producing ability,
wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,
wherein any of the following mutations (i), (i'), (i''), or (ii) is introduced into the mutant-type yggB gene:
(i) deletion of a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;
(i') insertion of an insertion sequence or a transposon into a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;
(i'') a mutation in which proline in a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62 is substituted with another amino acid;
or
(ii) substitution, deletion, or insertion of one to five amino acids in a region selected from the group consisting of amino acid numbers 1 to 23, 86 to 108, and 110 to 132 of an amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.
[Claim 4] (Corrected Invention 2-2)
A coryneform bacterium having an L-glutamic acid-producing ability,
wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,
wherein the mutation in the mutant-type yggB gene is a mutation in which alanine at position 100 is substituted with threonine, and/or alanine at position 111 is substituted with threonine or valine, in the amino acid sequence SEQ ID NO: 6, 62, 68, 84, or 85.
[Claim 6] (Corrected Invention 2-3)
The coryneform bacterium according to any of Claims 1, 2, 4, and 5, wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (a) to (n):
(a) DNA encoding an amino acid sequence of SEQ ID NO: 8;
(b) DNA encoding a protein having an amino acid sequence in which one to five amino

acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 8, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(c) DNA encoding an amino acid sequence of SEQ ID NO: 20;
(d) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 20, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(e) DNA encoding an amino acid sequence of SEQ ID NO: 22;
(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;
(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(i) DNA encoding an amino acid sequence of SEQ ID NO: 64;
(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(k) DNA encoding an amino acid sequence of SEQ ID NO: 70;
(l) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 70, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(m) DNA encoding an amino acid sequence of SEQ ID NO: 74; and
(n) DNA encoding a protein having an amino acid sequence in which one to five amino

acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 74, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin.

[Claim 10] (Corrected Invention 2-4)

The coryneform bacterium according to any of Claims 1, 2, and 4 to 9, wherein the coryneform bacterium belongs to the genus *Corynebacterium* or the genus *Brevibacterium*.

[Claim 11] (Corrected Invention 2-5)

A process for producing L-glutamic acid, comprising culturing a coryneform bacterium according to any of Claims 1, 2, and 4 to 10 in a medium so as to cause production and accumulation of L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

Attachment 5-3 "Re-corrected Claims (Patent 2)"

[Claim 4] (Re-corrected Invention 2-2) (the same contents as Corrected Invention 2-2)
A coryneform bacterium having an L-glutamic acid-producing ability,
wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,
wherein the mutation in the mutant-type yggB gene is a mutation in which alanine at position 100 is substituted with threonine, and/or alanine at position 111 is substituted with threonine or valine, in the amino acid sequence SEQ ID NO: 6, 62, 68, 84, or 85.
[Claim 13] (Re-corrected Invention 2-3)
The coryneform bacterium according to Claim 4, wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (e) to (j):
(e) DNA encoding an amino acid sequence of SEQ ID NO: 22;
(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;
(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(i) DNA encoding an amino acid sequence of SEQ ID NO: 64; and
(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin.
[Claim 14] (Re-corrected Invention 2-6)
A process for producing L-glutamic acid, comprising culturing a coryneform bacterium according to Claim 13 in a medium so as to cause production and accumulation of L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

End

Attachment 5-4 "Division into Constituent Features (Invention 2)"

Invention 2-1 (Claim 1)

2-A A coryneform bacterium having an L-glutamic acid-producing ability,

2-B wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,

2-C wherein any of the following mutations (i), (i'), (i''), or (ii) is introduced into the mutant-type yggB gene:

(i) deletion of a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;

(i') insertion of an insertion sequence or a transposon into a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;

(i'') a mutation in which proline in a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62 is substituted with another amino acid;

or

(ii) substitution, deletion, or insertion of one or several amino acids in a region selected from the group consisting of amino acid numbers 1 to 23, 86 to 108, and 110 to 132 of an amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.

Invention 2-2 (Claim 4)

2-E The coryneform bacterium according to Claim 1,

2-D wherein the mutation (ii) is a mutation in which alanine at position 100 and/or alanine at position 111 is substituted with another amino acid, in the amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.

Invention 2-3 (Claim 6)

2-F-2 The coryneform bacterium according to any of Claims 1 to 5,

2-F-1 wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (a) to (n):

2-F-3 (a) DNA encoding an amino acid sequence of SEQ ID NO: 8;
(b) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 8, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(c) DNA encoding an amino acid sequence of SEQ ID NO: 20;
(d) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 20, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(e) DNA encoding an amino acid sequence of SEQ ID NO: 22;
(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;
(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(i) DNA encoding an amino acid sequence of SEQ ID NO: 64;
(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(k) DNA encoding an amino acid sequence of SEQ ID NO: 70;

(l) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 70, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(m) DNA encoding an amino acid sequence of SEQ ID NO: 74; and
(n) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 74, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin.

Invention 2-4 (Claim 10)

2-G The coryneform bacterium according to any of Claims 1 to 9, wherein the coryneform bacterium belongs to the genus *Corynebacterium* or the genus *Brevibacterium*.

Invention 2-5 (Claim 11)

2-J A process for producing L-glutamic acid,

2-H comprising culturing a coryneform bacterium according to any of Claims 1 to 10 in a medium

2-I so as to cause production and accumulation of L-glutamic acid in the medium or the bacterium, and collecting the L-glutamic acid from the medium or the bacterium.

End

Attachment 5-5 "Division into Constituent Features (Corrected Invention 2)"

Corrected Invention 2-1 (Claim 1)

2-A A coryneform bacterium having an L-glutamic acid-producing ability,

2-B wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,

2-C' wherein any of the following mutations (i), (i'), (i''), or (ii) is introduced into the mutant-type yggB gene:

(i) deletion of a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;

(i') insertion of an insertion sequence or a transposon into a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62;

(i'') a mutation in which proline in a region of amino acid numbers 419 to 533 of an amino acid sequence of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62 is substituted with another amino acid;

or

(ii) substitution, deletion, or insertion of <u>one to five</u> amino acids in a region selected from the group consisting of amino acid numbers 1 to 23, 86 to 108, and 110 to 132 of an amino acid sequence of SEQ ID NO: 6, 62, 68, 84, or 85.

Corrected Invention 2-2 (Claim 4)

2-E'-1 <u>A coryneform bacterium having an L-glutamic acid-producing ability,</u>

2-E'-2 <u>wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain,</u>

2-D' <u>wherein the mutation in the mutant-type yggB gene is a mutation in which alanine at position 100 is substituted with threonine, and/or alanine at position 111 is substituted with threonine or valine, in the amino acid sequence SEQ ID NO: 6, 62, 68, 84, or 85</u>.

Corrected Invention 2-3 (Claim 6)

2-F'-2 The coryneform bacterium according to any of Claims 1, 2, 4, and 5,

2-F-1 wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (a) to (n):

2-F-3 (a) DNA encoding an amino acid sequence of SEQ ID NO: 8;

(b) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 8, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(c) DNA encoding an amino acid sequence of SEQ ID NO: 20;

(d) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 20, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(e) DNA encoding an amino acid sequence of SEQ ID NO: 22;

(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;

(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(i) DNA encoding an amino acid sequence of SEQ ID NO: 64;

(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the

coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(k) DNA encoding an amino acid sequence of SEQ ID NO: 70;
(l) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 70, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;
(m) DNA encoding an amino acid sequence of SEQ ID NO: 74; and
(n) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 74, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin.

Corrected Invention 2-4 (Claim 10)

2-G' The coryneform bacterium according to any of Claims 1, 2, and 4 to 9, wherein the coryneform bacterium belongs to the genus *Corynebacterium* or the genus *Brevibacterium*.

Corrected Invention 2-5 (Claim 11)

2-J A process for producing L-glutamic acid,
2-H' comprising culturing a coryneform bacterium according to any of Claims 1, 2, and 4 to 10 in a medium
2-I' so as to cause production and accumulation of L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

End

Attachment 5-6 "Division into Constituent Features (Re-corrected Invention 2)"

Re-corrected Invention 2-2 (Claim 4) (the same contents as Corrected Invention 2-2)

2-E'-1 A coryneform bacterium having an L-glutamic acid-producing ability,

2-E'-2 wherein a mutant-type yggB gene is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain

2-D' wherein the mutation in the mutant-type yggB gene is a mutation in which alanine at position 100 is substituted with threonine, and/or alanine at position 111 is substituted with threonine or valine, in the amino acid sequence SEQ ID NO: 6, 62, 68, 84, or 85.

Re-corrected Invention 2-3 (Claim 13)

2-F"-2 The coryneform bacterium according to Claim 4,

2-F'-1 wherein the mutant-type yggB gene is a mutant-type yggB gene selected from the group consisting of the following (e) to (j):

2-F'-3 (e) DNA encoding an amino acid sequence of SEQ ID NO: 22;

(f) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 22, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(g) DNA encoding an amino acid sequence of SEQ ID NO: 24;

(h) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 24, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium is cultured in a medium containing an excess of biotin;

(i) DNA encoding an amino acid sequence of SEQ ID NO: 64; and

(j) DNA encoding a protein having an amino acid sequence in which one to five amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 64, wherein the DNA is introduced into the coryneform bacterium, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced when the coryneform bacterium

is cultured in a medium containing an excess of biotin.

Re-corrected Invention 2-6 (Claim 14)

2-M A process for producing L-glutamic acid,

2-K comprising culturing a coryneform bacterium according to Claim 13 in a medium

2-L so as to cause production and accumulation of L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

End

Attachment 6-1 "Comparison between Defendant's Production Processes 1 and 3 and Invention 1"

1. Regarding Defendant's Production Process 1

As mentioned below, Defendant's Production Process 1 falls within the technical scopes of all of Inventions 1-1 through 1-4.

(1) Comparison with Invention 1-1

A. Regarding Constituent Feature 1-A-1

The Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TTGTCA sequence is introduced into the -35 region of the promoter sequence of the GDH gene, which is a gene on a chromosome, and therefore it fulfills Constituent Feature 1-A-1.

B. Regarding Constituent Feature 1-A-2

The Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TATAAT sequence is introduced into the -10 region of the promoter sequence of the GDH gene, which is a gene on a chromosome, and a TATAAT sequence is introduced into the -10 region of the promoter sequence of the CS gene, which is a gene on a chromosome.

Accordingly, Defendant's Production Process 1 fulfills Constituent Feature 1-A-2.

C. Regarding Constituent Feature 1-A-3

As mentioned in A. and B. above, the Defendant's Production Process 1 uses *Corynebacterium glutamicum*, which is a coryneform bacterium, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-A-3.

D. Regarding Constituent Feature 1-B

The Defendant's Production Process 1 uses the fermentation method comprising the steps of culturing "*Corynebacterium glutamicum*," which is a coryneform bacterium, in a liquid medium containing carbon sources, etc. to form and accumulate glutamic acid, and collecting glutamic acid from the medium by a separation operation.

Accordingly, Defendant's Production Process 1 fulfills Constituent Feature 1-B.

E. Summary

As mentioned in A. through D. above, Defendant's Production Process 1 fulfills Constituent Features 1-A-1 through 1-A-3 and 1-B, and therefore it falls within the technical scope of Invention 1-1.

(2) Comparison with Invention 1-2

A. Regarding Constituent Features 1-C-1 and 1-C-2

As mentioned in (1) A. and B. above, Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TTGTCA sequence is introduced into the -35 region and a TATAAT sequence is introduced into the -10 region of the promoter sequence of the GDH gene, which is a gene on a chromosome of the coryneform bacterium.

Accordingly, Defendant's Production Process 1 fulfills Constituent Features 1-C-1 and 1-C-2.

B. Regarding Constituent Feature 1-D

As mentioned in 1. (1) above, Defendant's Production Process 1 uses the method described in Claim 1 of Patent 1, and therefore it fulfills Constituent Feature 1-D.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 1 fulfills Constituent Features 1-C-1, 1-C-2, and 1-D, and therefore it falls within the technical scope of Invention 1-2.

(3) Comparison with Invention 1-3

A. Regarding Constituent Features 1-E-1 and 1-E-2

As mentioned in (1) B. above, in Defendant's Production Process 1, the promoter sequence of the GDH gene, which is a gene on a chromosome of "*Corynebacterium glutamicum*," which is a coryneform bacterium, has a TATAAT sequence in the -10 region, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-E-2.

B. Regarding Constituent Feature 1-F

As mentioned in (1) and (2) above, Defendant's Production Process 1 uses the method described in Claim 2 of Patent 1, and therefore it fulfills Constituent Feature 1-F.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 1 fulfills Constituent Features 1-E-2 and 1-F, and therefore it falls within the technical scope of Invention 1-3.

(4) Comparison with Invention 1-4

A. Regarding Constituent Features 1-G-1 and 1-G-2

As mentioned in (1) B. above, in Defendant's Production Process 1, the promoter sequence of the CS gene, which is a gene on a chromosome of a coryneform bacterium, has a TATAAT sequence in the -10 region, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-G-2.

B. Regarding Constituent Feature 1-H

As mentioned in (1) above, Defendant's Production Process 1 uses the method described in Claim 1 of Patent 1, and therefore it fulfills Constituent Feature 1-H.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 1 fulfills Constituent Features 1-G-2 and 1-H, and therefore it falls within the technical scope of Invention 1-4.

2 Regarding Defendant's Production Process 3

In relation to Invention 1, Defendant's Production Process 3 differs from Defendant's Production Process 1 in respect to the promoter sequence of the GDH gene of *Corynebacterium glutamicum* used in the process, and it is the same as Defendant's Production Process 1 in other respects.

As mentioned below, Defendant's Production Process 3 falls within the technical scope of Inventions 1-1 and 1-4.

(1) Comparison with Invention 1-1

A. Regarding Constituent Features 1-A-1 and 1-A-2

Defendant's Production Process 3 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TATAAT sequence is introduced into the -10 region of the promoter sequence of the CS gene, which is a gene on a chromosome of the coryneform bacterium.

Accordingly, Defendant's Production Process 3 fulfills Constituent Feature 1-A-2.

B. Regarding Constituent Features 1-A-3 and 1-B

As is the case with Defendant's Production Process 1, Defendant's Production Process 3 fulfills Constituent Features 1-A-3 and 1-B.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 3 fulfills Constituent Features 1-A-2, 1-A-3, and 1-B, and therefore it falls within the technical scope of Invention 1-1.

(2) Comparison with Invention 1-4

A. Regarding Constituent Features 1-G-1 and 1-G-2

As is the case with Defendant's Production Process 1, in Defendant's Production Process 3, the promoter sequence of the CS gene, which is a gene on a chromosome of a coryneform bacterium, has a TATAAT sequence in the -10 region, and therefore it fulfills Constituent Feature 1-G-2.

B. Regarding Constituent Feature 1-H

As is the case with Defendant's Production Process 1, Defendant's Production Process 3 fulfills Constituent Feature 1-H.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 3 fulfills Constituent Features 1-G-2 and 1-H, and therefore it falls within the technical scope of Invention 1-4.

Attachment 6-2 "Comparison between Defendant's Production Processes 1 and 3 and Corrected Invention 1"

1. Regarding Defendant's Production Process 1

As mentioned below, Defendant's Production Process 1 falls within the technical scopes of all of Corrected Invention 1-1 through 1-3.

(1) Comparison with Corrected Invention 1-1

A. Regarding Constituent Feature 1-A'-1

Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TTGTCA sequence is introduced into the -35 region and a TATAAT sequence is introduced into the -10 region of the promoter sequence of the GDH gene, which is a gene on a chromosome, and therefore it fulfills Constituent Feature 1-A'-1.

B. Regarding Constituent Feature 1-A'-2

The Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TATAAT sequence is introduced into the -10 region of the promoter sequence of the CS gene, which is a gene on a chromosome, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-A'-2.

C. Regarding Constituent Feature 1-A-3

As mentioned in A. and B. above, the Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-A-3.

D. Regarding Constituent Feature 1-B'

The Defendant's Production Process 1 uses the fermentation method comprising the steps of culturing "*Corynebacterium glutamicum*," which is a coryneform bacterium, in a liquid medium containing carbon sources, etc. to form and accumulate glutamic acid, and collecting glutamic acid from the medium by a separation operation, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-B'.

E. Summary

As mentioned in A. through D. above, Defendant's Production Process 1 fulfills Constituent Features 1-A'-1, 1-A'-2, 1-A-3, and 1-B', and therefore it falls within the technical scope of Corrected Invention 1-1.

(2) Comparison with Corrected Invention 1-2

A. Regarding Constituent Features 1-C'-1 and 1-C'-2

Defendant's Production Process 1 uses *Corynebacterium glutamicum* in which the

sequence mentioned in (1) A. above is introduced into the promoter sequence of the GDH gene, which is a gene on a chromosome, and therefore Defendant's Production Process 1 fulfills Constituent Features 1-C'-1 and 1-C'-2.

B. Regarding Constituent Feature 1-D

As mentioned in (1) above, Defendant's Production Process 1 uses the method described in Claim 1 of Patent 1 after Correction 1, and therefore it fulfills Constituent Feature 1-D.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 1 fulfills Constituent Features 1-C'-1, 1-C'-2, and 1-D, and therefore it falls within the technical scope of Corrected Invention 1-2.

(3) Comparison with Corrected Invention 1-3

A. Regarding Constituent Feature 1-G'

As mentioned in (1) B. above, in Defendant's Production Process 1, the promoter sequence of the CS gene, which is a gene on a chromosome of a coryneform bacterium, has a TATAAT sequence in the -10 region, and therefore it fulfills Constituent Feature 1-G'.

B. Regarding Constituent Feature 1-G"

Defendant's Production Process 1 uses *Corynebacterium glutamicum* in which the sequence mentioned in (1) A. above is introduced into the promoter sequence of the GDH gene, which is a gene on a chromosome, and therefore Defendant's Production Process 1 fulfills Constituent Feature 1-G".

C. Regarding Constituent Feature 1-H

As mentioned in (1) above, Defendant's Production Process 1 uses the method described in Claim 1 of Patent 1 after Correction 1, and therefore it fulfills Constituent Feature 1-H.

D. Summary

As mentioned in A. through C. above, Defendant's Production Process 1 fulfills Constituent Features 1-G', 1-G", and 1-H, and therefore it falls within the technical scope of Corrected Invention 1-3.

2. Regarding Defendant's Production Process 3

In relation to Corrected Invention 1, Defendant's Production Process 3 differs from Defendant's Production Process 1 in respect to the promoter sequence of the GDH gene of *Corynebacterium glutamicum* used in the process, and it is the same as Defendant's

Production Process 1 in other respects.

As mentioned below, Defendant's Production Process 3 falls within the technical scope of Corrected Invention 1-1.

(1) Comparison with Corrected Invention 1-1

A. Regarding Constituent Features 1-A'-1 and 1-A'-2

The Defendant's Production Process 3 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium, in which a TATAAT sequence is introduced into the -10 region of the promoter sequence of the CS gene, which is a gene on a chromosome, and therefore Defendant's Production Process 3 fulfills Constituent Feature 1-A'-2.

B. Regarding Constituent Features 1-A-3 and 1-B'

As is the case with Defendant's Production Process 1, Defendant's Production Process 3 fulfills Constituent Features 1-A-3 and 1-B'.

C. Summary

As mentioned in A. and B. above, Defendant's Production Process 3 fulfills Constituent Features 1-A'-2, 1-A-3, and 1-B', and therefore it falls within the technical scope of Corrected Invention 1-1.

(2) Comparison with Corrected Invention 1-3

Defendant's Production Process 3 does not fulfill Constituent Feature 1-G", and therefore it does not fall within the technical scope of Corrected Invention 1-3.

Attachment 7-1 "Comparison between Defendant's Production Processes 1 through 3 and Invention 2"

1. Regarding Defendant's Production Process 1

As mentioned below, the bacterium used in Defendant's Production Process 1 falls within the technical scopes of Inventions 2-1 (Claim 1), 2-2 (Claim 4), 2-3 (Claim 6), and 2-4 (Claim 10), and therefore Defendant's Production Process 1, which uses that bacterium, falls within the technical scope of Invention 2-5 (Claim 11).

(1) Comparison between the bacterium used in Defendant's Production Process 1 and Invention 2-1

A. Regarding Constituent Feature 2-A

Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium having a glutamic acid-producing ability, and therefore the bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-A.

B. Regarding Constituent Feature 2-B

Defendant's Production Process 1 uses *Corynebacterium glutamicum* into which a mutant-type yggB gene, wherein the amino acid at position 100 is substituted with threonine in the amino acid sequence of the yggB gene, is introduced, and thereby the L-glutamic acid-producing ability of the bacterium is enhanced as compared to a non-modified strain, and therefore the bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-B.

C. Regarding Constituent Feature 2-C

The mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced (the mutation mentioned in Constituent Feature 2-C (ii)).

Accordingly, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-C.

D. Summary

As mentioned in A. through C. above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-A through 2-C, and therefore it constitutes the coryneform bacterium described in Claim 1 of Patent 2.

(2) Comparison between the bacterium used in Defendant's Production Process 1 and Invention 2-2

A. Regarding Constituent Feature 2-D

As mentioned in (1) C. above, the mutant-type yggB gene introduced into *Corynebacterium glutamicum* used in Defendant's Production Process 1 is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced, and therefore it fulfills Constituent Feature 2-D.

B. Regarding Constituent Feature 2-E

As mentioned in (1) above, the bacterium used in Defendant's Production Process 1 is *Corynebacterium glutamicum*, which constitutes the coryneform bacterium described in Claim 1 of Patent 2, and therefore it fulfills Constituent Feature 2-E.

C. As mentioned in A. and B. above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-D and 2-E of Invention 2-2, and therefore it constitutes the coryneform bacterium described in Claim 4 of Patent 2.

(3) Comparison between the bacterium used in Defendant's Production Process 1 and Invention 2-3

A. Regarding Constituent Features 2-F-1 and 2-F-3

As mentioned in (1) C. above, the mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium that is used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced, and it encodes an amino acid sequence of SEQ ID NO: 22 in Description 2 (the amino acid sequence mentioned in 2-F-3 (e)).

Accordingly, this bacterium fulfills Constituent Features 2-F-1 and 2-F-3.

B. Regarding Constituent Feature 2-F-2

As mentioned in (1) and (2) above, the coryneform bacterium used in Defendant's Production Process 1 constitutes the coryneform bacterium described in Claims 1 and 4 of Patent 2, and therefore it fulfills Constituent Feature 2-F-2.

C. Summary

As mentioned above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-F-1, 2-F-2, and 2-F-3 of Invention 2-3, and therefore it constitutes the coryneform bacterium described in Claim 6 of Patent 2.

(4) Comparison between the bacterium used in Defendant's Production Process 1 and Invention 2-4

A. Regarding Constituent Feature 2-G

As mentioned in (1) through (3) above, the coryneform bacterium used in Defendant's Production Process 1 is "*Corynebacterium glutamicum*," which constitutes the coryneform bacterium described in Claims 1, 4, and 6 of Patent 2, and this bacterium is a coryneform bacterium which belongs to the genus *Corynebacterium*, and therefore it fulfills Constituent Feature 2-G.

B. Summary

As mentioned above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-G of Invention 2-4, and therefore it constitutes the coryneform bacterium described in Claim 10 of Patent 2.

(5) Comparison between Defendant's Production Process 1 and Invention 2-5

A. Regarding Constituent Feature 2-H

As mentioned in (1) through (4) above, Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which constitutes the coryneform bacterium described in Claims 1, 4, 6, and 10 of Patent 2, and this bacterium is cultured in a medium containing various ingredients including carbon sources.

Accordingly, Defendant's Production Process 1 fulfills Constituent Feature 2-H.

B. Regarding Constituent Feature 2-I

Defendant's Production Process 1 collects glutamic acid from fermented liquid that serves as the medium in which *Corynebacterium glutamicum*, which is a coryneform bacterium, was cultured, and therefore it fulfills Constituent Feature 2-I.

C. Regarding Constituent Feature 2-J

Defendant's Production Process 1 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-J.

D. As mentioned in A. through C. above, Defendant's Production Process 1 fulfills Constituent Features 2-H through 2-J, and therefore it falls within the technical scope of Invention 2-5.

2 Regarding Defendant's Production Processes 2 and 3

Defendant's Production Processes 2 and 3 use *Corynebacterium glutamicum* into which the same mutant-type yggB gene as that in Defendant's Production Process 1 is introduced, and therefore in relation to Invention 2, they fulfill the respective Constituent Features in the same manner as Defendant's Production Process 1.

Accordingly, both Defendant's Production Processes 2 and 3 fall within the technical scope of Invention 2-5.

Attachment 7-2 "Comparison between Defendant's Production Processes 1 through 3 and Corrected Invention 2"

1. Regarding Defendant's Production Process 1

As mentioned below, the bacterium used in Defendant's Production Process 1 falls within the technical scopes of Corrected Inventions 2-1 (Claim 1), 2-2 (Claim 4), 2-3 (Claim 6), and 2-4 (Claim 10), and therefore Defendant's Production Process 1, which uses that bacterium, falls within the technical scope of Corrected Invention 2-5 (Claim 11).

(1) Comparison between the bacterium used in Defendant's Production Process 1 and Invention 2-1

A. Regarding Constituent Feature 2-A

Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium having a glutamic acid-producing ability, and therefore the bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-A.

B. Regarding Constituent Feature 2-B

Defendant's Production Process 1 uses *Corynebacterium glutamicum* into which a mutant-type yggB gene, wherein the amino acid at position 100 is substituted with threonine in the amino acid sequence of the yggB gene, is introduced, and thereby the L-glutamic acid-producing ability of the bacterium is enhanced as compared to a non-modified strain, and therefore the bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-B.

C. Regarding Constituent Feature 2-C'

The mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium that is used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced (the mutation mentioned in Constituent Feature 2-C' (ii)).

Accordingly, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-C'.

D. Summary

As mentioned in A. through C. above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-A, 2-B, and 2-C', and therefore it constitutes the coryneform bacterium described in Claim 1 of Patent 2 after Correction 2.

(2) Comparison between the bacterium used in Defendant's Production Process 1 and

Corrected Invention 2-2

A. Regarding Constituent Features 2-E'-1 and 2-E'-2

As mentioned in (1) A. and B. above, Defendant's Production Process 1 uses *Corynebacterium glutamicum* into which a mutant-type yggB gene is introduced, and therefore the bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-E'-1 and 2-E'-2.

B. Regarding Constituent Feature 2-D'

As mentioned in (1) C. above, the mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium that is used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced, and therefore the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-D'.

C. As mentioned in A. and B. above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-E'-1, 2-E'-2, and 2-D' of Corrected Invention 2-2, and therefore it constitutes the coryneform bacterium described in Claim 4 of Patent 2 after Correction 2.

(3) Comparison between the bacterium used in Defendant's Production Process 1 and Corrected Invention 2-3

A. Regarding Constituent Features 2-F-1 and 2-F-3

As mentioned in (1) C. above, the mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium that is used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced, and it encodes an amino acid sequence of SEQ ID NO: 22 in Description 2 (the amino acid sequence mentioned in 2-F-3 (e)).

Accordingly, this bacterium fulfills Constituent Features 2-F-1 and 2-F-3.

B. Regarding Constituent Feature 2-F'-2

As mentioned in (1) and (2) above, the coryneform bacterium used in Defendant's Production Process 1 constitutes the coryneform bacterium described in Claims 1 and 4 of Patent 2 after Correction 2, and therefore it fulfills Constituent Feature 2-F'-2.

C. Summary

As mentioned above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-F-1, 2-F'-2, and 2-F-3 of Corrected Invention 2-3, and therefore it constitutes the coryneform bacterium described in Claim 6 after

Correction 2.

(4) Comparison between the bacterium used in Defendant's Production Process 1 and Corrected Invention 2-4

A. Regarding Constituent Feature 2-G'

As mentioned in (1) through (3) above, the coryneform bacterium used in Defendant's Production Process 1 is "*Corynebacterium glutamicum*," which constitutes the coryneform bacterium described in Claims 1, 4, and 6 of Patent 2 after Correction 2, and this bacterium is a coryneform bacterium which belongs to the genus *Corynebacterium*, and therefore it fulfills Constituent Feature 2-G'.

B. Summary

As mentioned above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Feature 2-G' of Invention 2-4, and therefore it constitutes the coryneform bacterium described in Claim 10 of Patent 2.

(5) Comparison between Defendant's Production Process 1 and Corrected Invention 2-5

A. Regarding Constituent Feature 2-H'

As mentioned in (1) through (4) above, Defendant's Production Process 1 uses "*Corynebacterium glutamicum*," which constitutes the coryneform bacterium described in Claims 1, 4, 6, and 10 of Patent 2 after Correction 2, and this bacterium is cultured in a medium containing various ingredients including carbon sources.

Accordingly, Defendant's Production Process 1 fulfills Constituent Feature 2-H'.

B. Regarding Constituent Feature 2-I'

Defendant's Production Process 1 collects glutamic acid from fermented liquid that serves as the medium in which *Corynebacterium glutamicum*, which is a coryneform bacterium, was cultured, and therefore it fulfills Constituent Feature 2-I'.

C. Regarding Constituent Feature 2-J

Defendant's Production Process 1 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-J.

D. As mentioned in A. through C. above, Defendant's Production Process 1 fulfills Constituent Features 2-H', 2-I', and 2-J, and therefore it falls within the technical scope of Corrected Invention 2-5.

2. Regarding Defendant's Production Processes 2 and 3

Defendant's Production Processes 2 and 3 use *Corynebacterium glutamicum* into which the same mutant-type yggB gene as that in Defendant's Production Process 1 is

introduced, and therefore in relation to Corrected Invention 2, they fulfill the respective Constituent Features in the same manner as Defendant's Production Process 1.

Accordingly, both Defendant's Production Processes 2 and 3 fall within the technical scope of Corrected Invention 2-5.

Attachment 7-3 "Comparison between Defendant's Production Processes 1 through 3 and Re-corrected Invention 2"

1. Regarding Defendant's Production Process 1

As mentioned below, the bacterium used in Defendant's Production Process 1 falls within the technical scopes of Re-corrected Inventions 2-2 (Claim 4) and 2-3 (Claim 13), and therefore Defendant's Production Process 1, which uses that bacterium, falls within the technical scope of Re-corrected Invention 2-6 (Claim 14).

(1) Comparison between the bacterium used in Defendant's Production Process 1 and Re-corrected Invention 2-2

While the contents of Re-corrected Invention 2-2 are the same as the contents of Corrected Invention 2-2, as mentioned in 1. (2) of Attachment 7-2 "Comparison between Defendant's Production Processes 1 through 3 and Corrected Invention 2," the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-E'-1, 2-E'-2, and 2-D', and therefore, it constitutes the coryneform bacterium described in Claim 4 of Patent 2 after Correction 2.

(2) Comparison between the bacterium used in Defendant's Production Process 1 and Re-corrected Invention 2-3

A. Regarding Constituent Features 2-F'-1 and 2-F'-3

The mutant-type yggB gene introduced into *Corynebacterium glutamicum*, which is a coryneform bacterium that is used in Defendant's Production Process 1, is one into which a mutation wherein alanine at position 100 in the amino acid sequence of SEQ ID NO: 6 in Description 2 is substituted with threonine is introduced, and it encodes an amino acid sequence of SEQ ID NO: 22 in Description 2 (the amino acid sequence mentioned in 2-F'-3 (e)).

Accordingly, this bacterium fulfills Constituent Features 2-F'-1 and 2-F'-3.

B. Regarding Constituent Feature 2-F"-2

As mentioned in (1) above, the coryneform bacterium used in Defendant's Production Process 1 constitutes the coryneform bacterium described in Claim 4 of Patent 2 after the re-correction, and therefore it fulfills Constituent Feature 2-F"-2.

C. Summary

As mentioned in A. and B. above, the coryneform bacterium used in Defendant's Production Process 1 fulfills Constituent Features 2-F'-1, 2-F"-2, and 2-F'-3 of Re-corrected Invention 2-3, and therefore it constitutes the coryneform bacterium described in Claim 13 of Patent 2 after the re-correction.

(3) Comparison between Defendant's Production Process 1 and Re-corrected Invention 2-6

A. Regarding Constituent Feature 2-K

As mentioned in (2) above, Defendant's Production Process 1 uses *Corynebacterium glutamicum*, which constitutes the coryneform bacterium described in Claim 13 of Patent 2 after the re-correction, and this bacterium is cultured in a medium containing various ingredients including carbon sources.

Accordingly, Defendant's Production Process 1 fulfills Constituent Feature 2-K.

B. Regarding Constituent Feature 2-L

Defendant's Production Process 1 collects glutamic acid from fermented liquid that serves as the medium in which *Corynebacterium glutamicum*, which is a coryneform bacterium, was cultured, and therefore it fulfills Constituent Feature 2-L.

C. Regarding Constituent Feature 2-M

Defendant's Production Process 1 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-M.

D. As mentioned in A. through C. above, Defendant's Production Process 1 fulfills Constituent Features 2-K, 2-L, and 2-M, and therefore it falls within the technical scope of Re-corrected Invention 2-6.

2. Regarding Defendant's Production Processes 2 and 3

Defendant's Production Processes 2 and 3 use *Corynebacterium glutamicum* into which the same mutant-type yggB gene as that in Defendant's Production Process 1 is introduced, and therefore in relation to Re-corrected Invention 2, they fulfill the respective Constituent Features in the same manner as Defendant's Production Process 1.

Accordingly, both Defendant's Production Processes 2 and 3 fall within the technical scope of Re-corrected Invention 2-6.

Attachment 8-1 "Comparison between Defendant's Production Process 4 and Invention 2"

1. Comparison between the strains used in Defendant's Production Process 4 and Inventions 2-1, 2-2, and 2-3

(1) Comparison between the strains used in Defendant's Production Process 4 and Invention 2-1

A. Regarding Constituent Feature 2-A

Defendant's Production Process 4 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium having a glutamic acid-producing ability, and therefore the strains used in Defendant's Production Process 4 fulfill Constituent Feature 2-A.

B. Regarding Constituent Feature 2-B

A mutant-type yggB gene, wherein alanine at position 98 is substituted with threonine (A98T mutation) and valine at position 241 is substituted with isoleucine (V241I mutation) in YggB of wild-type *Corynebacterium callunae*, is introduced into the strains used in Defendant's Production Process 4. In addition, the glutamic acid production amount has increased as compared to a non-modified strain, and therefore, the strains fulfill Constituent Feature 2-B.

C. Regarding Constituent Feature 2-C

The strains used in Defendant's Production Process 4 are those of *Corynebacterium glutamicum*, which is a coryneform bacterium, into which the mutant-type yggB gene mentioned in B. above is introduced. This mutant-type yggB gene does not coincide with the DNA encoding an amino acid sequence in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2 (the mutation mentioned in (ii)), and it is not one into which any of the other mutations prescribed in Constituent Feature 2-C is introduced.

Accordingly, the strains used in Defendant's Production Process 4 do not fulfill Constituent Feature 2-C.

(2) Comparison between the strains used in Defendant's Production Process 4 and Invention 2-2

A. Regarding Constituent Feature 2-D

Since the strains used in Defendant's Production Process 4 are as mentioned in (1) B. above, their mutation does not coincide with one in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2, and the strains are not those into which any of the other mutations prescribed in

Constituent Feature 2-D is introduced.

B. Regarding Constituent Feature 2-E

As mentioned in (1) above, the strains used in Defendant's Production Process 4 do not fulfill the part of Constituent Feature 2-E relating to Constituent Feature 2-C of Invention 2-1.

(3) Comparison between the strains used in Defendant's Production Process 4 and Invention 2-3

As mentioned in (1) B. above, the mutant-type yggB gene of the strains used in Defendant's Production Process 4 does not coincide with the DNA encoding an amino acid sequence in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2 (the amino acid sequence of SEQ ID NO: 22 in 2-F-3 (e)), and it is not one into which any of the other mutations prescribed in Constituent Feature 2-F-3 is introduced.

In addition, according to (1) and (2) above, it also does not fulfill Constituent Feature 2-F-2.

Accordingly, the strains used in Defendant's Production Process 4 do not fulfill Constituent Features 2-F-1, 2-F-2, and 2-F-3.

2. Comparison between Defendant's Production Process 4 and Invention 2-5

(1) Regarding Constituent Feature 2-H

In Defendant's Production Process 4, the coryneform bacterium used is cultured in a medium containing various ingredients including carbon sources.

Accordingly, if the strains used are equivalent to the coryneform bacterium described in Claim 6 (Invention 2-3), they fulfill Constituent Feature 2-H.

(2) Regarding Constituent Feature 2-I

Defendant's Production Process 4 collects glutamic acid from fermented liquid that serves as the medium in which the coryneform bacterium was cultured, and therefore it fulfills Constituent Feature 2-I.

(3) Regarding Constituent Feature 2-J

Defendant's Production Process 4 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-J.

Attachment 8-2 "Comparison between Defendant's Production Process 4 and Corrected Invention 2"

1. Comparison between the strains used in Defendant's Production Process 4 and Corrected Inventions 2-2 and 2-3

(1) Comparison between the strains used in Defendant's Production Process 4 and Corrected Invention 2-2

A. Regarding Constituent Feature 2-E'-1

Defendant's Production Process 4 uses "*Corynebacterium glutamicum*," which is a coryneform bacterium having a glutamic acid-producing ability, and therefore the strains used in Defendant's Production Process 4 fulfill Constituent Feature 2-E'-1.

B. Regarding Constituent Feature 2-E'-2

A mutant-type yggB gene, wherein alanine at position 98 is substituted with threonine (A98T mutation) and valine at position 241 is substituted with isoleucine (V241I mutation) in YggB of wild-type *Corynebacterium callunae*, is introduced into the strains used in Defendant's Production Process 4. In addition, the glutamic acid production amount has increased as compared to a non-modified strain, and therefore, the strains fulfill Constituent Feature 2-E'-2.

C. Regarding Constituent Feature 2-D'

Since the strains used in Defendant's Production Process 4 are as mentioned in B. above, their mutation does not coincide with one in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2, and the strains are not those into which any of the other mutations prescribed in Constituent Feature 2-D' is introduced, and therefore the strains do not fulfill Constituent Feature 2-D'.

(2) Comparison between the strains used in Defendant's Production Process 4 and Corrected Invention 2-3

As mentioned in (1) B. above, the mutant-type yggB gene of the strains used in Defendant's Production Process 4 does not coincide with the DNA encoding an amino acid sequence in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2 (the amino acid sequence of SEQ ID NO: 22 in 2-F-3 (e)), and it is not one into which any of the other mutations prescribed in Constituent Feature 2-F-3 is introduced.

In addition, according to (1) above, it also does not fulfill Constituent Feature 2-F'-2.

Accordingly, the strains used in Defendant's Production Process 4 do not fulfill

Constituent Features 2-F-1, 2-F'-2, and 2-F-3.

2. Comparison between Defendant's Production Process 4 and Corrected Invention 2-5

(1) Regarding Constituent Feature 2-H'

In Defendant's Production Process 4, the coryneform bacterium used is cultured in a medium containing various ingredients including carbon sources.

Accordingly, if the strains used are equivalent to the coryneform bacterium described in Claim 6 after the correction (Corrected Invention 2-3), they fulfill Constituent Feature 2-H'.

(2) Regarding Constituent Feature 2-I'

Defendant's Production Process 4 collects glutamic acid from fermented liquid that serves as the medium in which the coryneform bacterium was cultured, and therefore it fulfills Constituent Feature 2-I'.

(3) Regarding Constituent Feature 2-J

Defendant's Production Process 4 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-J.

Attachment 8-3 "Comparison between Defendant's Production Process 4 and Re-corrected Invention 2"

1. Comparison between the strains used in Defendant's Production Process 4 and Re-corrected Inventions 2-2 and 2-3

(1) Comparison between the strains used in Defendant's Production Process 4 and Re-corrected Invention 2-2

Re-corrected Invention 2-2 is identical to Corrected Invention 2-2. Accordingly, as mentioned in 1. (1) of Attachment 8-2 "Comparison between Defendant's Production Process 4 and Corrected Invention 2," the strains used in Defendant's Production Process 4 fulfill Constituent Features 2-E'-1 and 2-E'-2, and do not fulfill Constituent Feature 2-D'.

(2) Comparison between the strains used in Defendant's Production Process 4 and Re-corrected Invention 2-3

The strains used in Defendant's Production Process 4 are those of *Corynebacterium glutamicum*, which is a coryneform bacterium, into which a mutant-type yggB gene, wherein alanine at position 98 is substituted with threonine (A98T mutation) and valine at position 241 is substituted with isoleucine (V241I mutation) in YggB of wild-type *Corynebacterium callunae*, is introduced. This mutant-type yggB gene does not coincide with the DNA encoding an amino acid sequence in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6 in Description 2 (the amino acid sequence of SEQ ID NO: 22 in 2-F'-3 (e)), and it is not one into which any of the other mutations prescribed in Constituent Feature 2-F'-3 is introduced.

In addition, according to (1) above, it also does not fulfill Constituent Feature 2-F"-2.

Accordingly, the strains used in Defendant's Production Process 4 do not fulfill Constituent Features 2-F'-1, 2-F"-2, and 2-F'-3.

2. Comparison between Defendant's Production Process 4 and Re-corrected Invention 2-6

(1) Regarding Constituent Feature 2-K

In Defendant's Production Process 4, the coryneform bacterium used is cultured in a medium containing various ingredients including carbon sources.

Accordingly, if the strains used are equivalent to the coryneform bacterium described in Claim 13 of Patent 2 after the re-correction (Re-corrected Invention 2-3), they fulfill Constituent Feature 2-K.

(2) Regarding Constituent Feature 2-L

Defendant's Production Process 4 collects glutamic acid from fermented liquid that serves as the medium in which the coryneform bacterium was cultured, and therefore it fulfills Constituent Feature 2-L.

(3) Regarding Constituent Feature 2-M

Defendant's Production Process 4 is a process for producing glutamic acid, and therefore it fulfills Constituent Feature 2-M.

Attachment 9 "Comparison Table of Allegations concerning Processes for Producing the MSG in Question"

	Defendant's allegations						Plaintiff's alternative claims	Plaintiff's principal claims
Period	No.	Strain No.	CS	GDH		ygg B		
			-10	-35	-10			
●(omitted)●	[i]		Unknown due to lack of data					
●(omitted)●	[ii]		TATAAT	TTGTCA	TATAAT	A100T*	Defendant's Production Process 1	
●(omitted)●	[iii]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 2	
●(omitted)●	[iv]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 2	
●(omitted)●	[v]	●(omitted)●	TATAAT	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 3	
●(omitted)●	[vi]	●(omitted)●	TATAAT	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 3	Defendant's Production Process 1
●(omitted)●	[vii]	●(omitted)●	TATAAT	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 3	
●(omitted)●	[viii]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 2	
●(omitted)●	[ix]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 2	
●(omitted)●	[x]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	A100T	Defendant's Production Process 2	
●(omitted)●	[xi]	●(omitted)	●(omitted)	●(omitted)	●(omitted)	A100T	Defendant's Production	

		●	●	●	●		Process 2	
●(omitted)●	[xii]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	**	Defendant's Production Process 4	
●(omitted)●	[xiii]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	**	Defendant's Production Process 4	
●(omitted)●	[xiv]	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	No mutation		

* Alanine at position 100 is substituted with threonine.

** ●(omitted)● in yggB of *Corynebacterium glutamicum*, and alanine at position 98 is substituted with threonine and valine at position 241 is substituted with isoleucine in yggB derived from *Corynebacterium callunae*.

Attachment 10 "List of Sales Values/Volumes"

1. Sales values (for 2011 through 2017)

	2011	2012	2013	2014	2015	2016	2017	Total
Portion sold by the Defendant	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●
Portion sold by CJ Indonesia	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●
Total sales value	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(Unit: 1,000 yen)

2. Sales volumes (for 2011 through 2017)

	2011	2012	2013	2014	2015	2016	2017	Total
Portion sold by the Defendant	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●
Portion sold by CJ Indonesia	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●
Total sales value	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(Unit: MT)

Attachment 11-1 "Allegations on the Value of Damages 1" (Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Overall Portion Sold by the Defendant, etc.)

[Plaintiff's allegations]

1. Outline of the allegations

As mentioned in No. 3, 15. [Plaintiff's allegations] (1) of the text, joint tort is found to be established between the Defendant and CJ Indonesia with regard to the overall portion sold by the Defendant, etc. Therefore, the Defendant is liable for tort due to the infringement of the respective Patent Rights with regard to the overall portion sold by the Defendant, etc.

Therefore, the total profits made by the Defendant, etc. in relation to the overall portion sold by the Defendant, etc. during the subject period (as mentioned in 2. below, in relation to Patent Right 2, the period on and after August 23, 2013 within that period) will be the value of damages to be compensated by the Defendant pursuant to Article 102, paragraph (2) of the Patent Act.

2. Profits made by the Defendant, etc. during the subject period

(1) Sales amount

The total sales amount relating to the portion sold by the Defendant, etc. during the subject period is ●(omitted)● yen (No. 3, 15. [Plaintiff's allegations] (2) of the text).

(2) Regarding the expenses borne by the Defendant, etc.

A. Sales volumes of the Defendant, etc.

The sales volumes of the Defendant, etc. during the subject period are as shown in No. 3, 15. [Plaintiff's allegations] (2) of the text.

B. Production cost for the MSG in question

(A) Production costs per ton

The production costs per ton (unit: U.S. dollars) of the MSG in question for 2009 through 2014 are as shown in the table below.

They indicate the total amounts of the fermentation material expenses, purification material expenses, utility expenses, and packaging expenses out of the expenses stated in material disclosed by the Defendant (Exhibit Otsu 107). Meanwhile, the expenses stated in that material as fixed expenses (labor, depreciation, and others) should not be included as expenses in calculating the profit under Article 102, paragraph (2) of the Patent Act.

As the costs for other years are not stated in that material, the costs for 2007 and 2008 are deemed to be the same amount as the cost for 2009, and the costs for 2015 through 2017 are deemed to be the same amount as the cost for 2014 in the calculation.

2009	2010	2011	2012	2013	2014
●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(B) Yen-dollar exchange rates

The exchange rates of U.S. dollars to yen during the subject period are as shown in the table below.

2007	2008	2009	2010	2011	2012
117.8	103.4	93.6	87.8	79.8	79.8
2013	2014	2015	2016	2017	
97.6	105.9	121.0	108.8	112.2	

(C) Production costs in yen

When the sales volumes mentioned in A. above are multiplied by the production costs per ton mentioned in (A) above and the exchange rates mentioned in (B) above, the production costs relating to the portion sold by the Defendant, etc. during the subject period come to a total of ●(omitted)● yen.

C. Expenses for international transportation

The expenses for international transportation (meaning the expenses for transportation from the factory in Indonesia to Japan; the same applies hereinafter) per ton of the MSG in question during the subject period are calculated as follows.

(A) The expenses for international transportation per ton in U.S. dollars are as shown in the table below (unit: U.S. dollars).

2011	2012	2013	2014	2015	2016	2017
●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(B) When the amounts of expenses in U.S. dollars mentioned in (A) above are converted into yen by using the exchange rates mentioned in B. (B) above, the average amount of expenses per ton will be ●(omitted)● yen, and it is reasonable to use that amount for calculating the expenses for international transportation during the subject period.

Accordingly, the expenses for international transportation for the sales volumes of the Defendant, etc. during the subject period mentioned in A. above come to a total of ●(omitted)● yen.

D. Domestic freight expenses and other selling, general, and administrative expenses

From among the selling, general, and administrative expenses of the Defendant stated in material disclosed by the Defendant (Exhibit Otsu 99), those that should be found to be expenses in calculating the profit under Article 102, paragraph (2) of the Patent Act are only the freight expenses and warehouse expenses shown in the table below. As the amounts of these expenses for 2007 through 2010 are unknown, they are deemed to be the same amounts as those for 2011 in the calculation.

Thus, the amount that should be found to be expenses as the selling, general, and administrative expenses relating to the portion sold by the Defendant, etc. during the subject period comes to a total of ●(omitted)● yen.

	2011	2012	2013	2014	2015	2016	2017
Freight expenses	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●
Warehouse expenses	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(unit: 1,000 yen)

E. Total amount of expenses

The total amount of expenses that should be taken into consideration for the portion sold by the Defendant, etc. during the subject period will be the sum of the amounts mentioned in B. through D. above, which comes to ●(omitted)● yen.

(3) Profits made by the Defendant, etc.

When the total amount of expenses mentioned in (2) E. above is deducted from the sales amount mentioned in (1) above, the amount of profits made by the Defendant, etc. in relation to the portion sold by the Defendant, etc. during the subject period comes to 4,994,885,970 yen.

3. Value of damages during the subject period for Patent Right 2 (August 23, 2013 through December 31, 2017)

(1) Sales amount

Of the sales amount relating to the portion sold by the Defendant, etc. for 2013, which is ●(omitted)● yen, the portion for the period from August 23, 2013 through the last day of that year is presumed to be ●(omitted)● yen, as calculated pro rata based on the number of days.

As the sales amounts for 2014 through 2017 are as shown in 1. of Attachment 10 "List of Sales Values/Volumes," the sales amount relating to the portion sold by the Defendant, etc. during the subject period for Patent Right 2 comes to ●(omitted)● yen.

(2) Regarding the expenses borne by the Defendant, etc.

When the expenses for 2013 are calculated for the period on and after August 23 of that year pro rata based on the number of days, and the expenses for 2014 through 2017 are calculated by the method stated in 2. (2) above, the amount that should be found to be expenses with regard to the portion sold by the Defendant, etc. during the subject period for Patent Right 2 will approximately be as follows:

A. Production cost ●(omitted)● yen

B. Expenses for international transportation ●(omitted)● yen

C. Domestic freight expenses, warehouse expenses, other selling, general, and administrative expenses ●(omitted)● yen

(3) Profits made by the Defendant, etc.

When the expenses mentioned in (2) above are deducted from the sales amount mentioned in (1) above, the amount of profits made by the Defendant, etc. in relation to the portion sold by the Defendant, etc. during the subject period for Patent Right 2 comes to 2,917,455,000 yen.

4. Relationship between the claims for compensation for damages caused by the infringement of the Patent Rights

Of the subject period, during the period from January 1, 2007 through August 22, 2013, only Patent Right 1 was infringed, and during the subject period for Patent Right 2 from August 23, 2013 onward, both of the Patent Rights were infringed.

In presuming the value of damages under Article 102, paragraph (2) of the Patent Act, with regard to the period in which only Patent Right 1 was infringed, the entire amount of the profits made by the Defendant, etc. during that period are found to be the damages caused by the infringement of Patent Right 1. With regard to the period in which both of the Patent Rights were infringed, the entire amount of profits made by the Defendant, etc. during that period are presumed to be the damages for the infringement of the respective Patent Rights, but in light of the purport of Article 102 of the Patent Act, the obligation of the Defendant to make payment remains within the scope of the entire profits made by the Defendant, etc. during that period.

With regard to the infringement of Patent Right 1, the Plaintiff is presumed to have sustained damages in an amount equal to the amount of profits made by the Defendant, etc. during the entire subject period, that is, the period during which the relevant patent right was infringed, which is 4,994,885,970 yen (mentioned in 2. above). With regard to the infringement of Patent Right 2, the Plaintiff is presumed to have sustained damages in an amount equal to the profits made by the Defendant, etc. during the subject period for Patent Right 2, that is, the period during which the relevant patent right was infringed, which is 2,917,455,000 yen (mentioned in 3. above), but because this amount overlaps with the damages caused by the infringement of Patent Right 1 during that period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of 2,917,455,000 yen, with regard to the infringement of the Patent Rights during that period.

As damages throughout the subject period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of 4,994,885,970 yen as mentioned in 2. above. In this lawsuit, the Plaintiff claims 900 million yen from that amount as partial claims for the damages.

5. Regarding grounds for the rebuttal of the presumption

(1) Regarding the rebuttal of the presumption based on the sale of competitive products (reduction of the amount according to the market share)

The Defendant alleges that the amount of damages should be reduced according to the market share of monosodium glutamate. However, in order to rebut the presumption under Article 102, paragraph (2) of the Patent Act, the Defendant needs to specifically prove the grounds for which the Plaintiff would not have been able to sell all of the portion sold by the Defendant, etc., and therefore the rebuttal of the presumption based on the market share is unreasonable.

The Defendant alleges that, out of the volume of monosodium glutamate imported from Indonesia into Japan, excluding the portion sold by the Defendant, etc., half was imported by the Plaintiff. In reality, however, most of that volume was imported by the Plaintiff, and the Defendant's calculation of the Plaintiff's share in the import volume is unreasonable. It is also unreasonable that, in the analysis of Exhibit Otsu 117, the volume of imports that were imported as glutamic acid and processed into monosodium glutamate in Japan (most of which were imports by the Plaintiff) are not taken into consideration.

(2) Regarding the rebuttal of the presumption based on the degree of contribution of the Patent Rights to damages

The Defendant makes allegations concerning the degree of contribution made by the Patents by strictly distinguishing the production processes, etc. of glutamic acid. However, as the Patents are processes for producing glutamic acid by using specific bacteria, and the Defendant, etc. sell glutamic acid produced by using those specific bacteria, it should be said that the degree of contribution of the Patents is 100%, and there is no ground regarding the rebuttal of the presumption based on the degree of contribution.

6. Regarding overlapped application of Article 102, paragraph (3) of the Patent Act for the rebutted part

Supposing that the rebuttal of the presumption of damages were to be allowed based on the existence of competitive products, the value of damages in an amount equivalent to royalties under Article 102, paragraph (3) of the Patent Act, as mentioned in Attachment 11-2, should be recognized for the rebutted part.

[Defendant's allegations]

1. Outline of the allegations

The Defendant disputes the Plaintiff's allegations concerning damages.

As mentioned in No. 3, 15. [Defendant's allegations] (1) of the text, the Defendant is not liable for compensation for damages with regard to the portion sold by CJ Indonesia. Therefore, when calculating the value of damages under Article 102, paragraph (2) of the

Patent Act, the portion sold by CJ Indonesia should not be taken into consideration.

In addition, as mentioned in No. 3, 15. [Defendant's allegations] (2) of the text, the Plaintiff's allegations concerning the sales values/volumes for 2007 through 2010 are unreasonable.

2. Regarding the expenses to be deducted

The Defendant disputes the Plaintiff's allegations concerning the amount of expenses to be deducted.

(1) Regarding selling, general, and administrative expenses

As selling, general, and administrative expenses (Exhibit Otsu 101), not only the freight expenses and warehouse expenses, but also the personnel expenses, service expenses, and other expenses should be taken into consideration. The total amount of selling, general, and administrative expenses for 2011 through 2017 is ●(omitted)● yen for the portion sold by the Defendant and ●(omitted)● yen for the portion sold by CJ Indonesia. Whereas Exhibit Otsu 101 only states the selling, general, and administrative expenses for 2011 onward, if the profits for 2007 through 2010 were to be taken into consideration, the selling, general, and administrative expenses for that period should also be estimated in a manner similar to that for the sales amounts, by a formula similar to that mentioned in No. 3, 15. [Defendant's allegations] (2) B. of the text, which will come to a total of ●(omitted)● yen for the portion sold by the Defendant and a total of ●(omitted)● yen for the portion sold by CJ Indonesia.

In accounting, the Defendant recorded the overall amounts of the freight expenses and warehouse expenses concerning the MSG in question by distributing them between the expenses for the portion sold by the Defendant and the expenses for the portion sold by CJ Indonesia (which had not actually occurred).

As for expenses that are common to the respective businesses, such as personnel expenses, the Defendant has proportionally distributed its overall amount of expenses among the respective departments according to the internal standards, and from among the expenses distributed to the department involved in the sale of the MSG in question, the expenses concerning the MSG in question are calculated according to the proportion of the sales of the MSG in question in the department's total sales. Moreover, on an accounting basis, expenses concerning the MSG in question are further distributed between those relating to the portion sold by the Defendant and the portion sold by CJ Indonesia. Also, while, from among these expenses, as personnel expenses include the personnel expenses of operation departments and those of indirect sections, at least the personnel expenses of operation departments shown in the table below depend on the workload of sales operations, so they should be deducted as expenses that became

additionally required in direct association with the sale of the MSG in question.

2011	2012	2013	2014	2015	2016	2017
●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●	●(omitted)●

(unit: 1,000 yen)

(2) Regarding purchasing expenses

When considering the profits for the portion sold by the Defendant, the expenses required for their purchase should be taken into consideration.

The total purchasing expenses for 2011 through 2017 is ●(omitted)● yen (Exhibits Otsu 99, 101). Whereas Exhibit Otsu 101 only states the selling, general, and administrative expenses for 2011 onward, if the profits for 2007 through 2010 were to be taken into consideration, the purchasing expenses for that period should also be estimated in a manner similar to that for the sales amounts, by a formula similar to that mentioned in No. 3, 15. [Defendant's allegations] (2) B. of the text, which will come to a total of ●(omitted)● yen.

3. Relationship between the claims for compensation for damages caused by the infringement of the Patent Rights

With regard to the value of damages presumed pursuant to Article 102, paragraph (2) of the Patent Act, the Plaintiff alleges that the value of damages will be the same between a case where one patent right has been infringed and a case where two patent rights have been infringed, as the value of damages will be equal to the total amount of the infringer's profits in both cases. However, if the number of patents infringed is smaller, the value of damages should also be smaller, so the Plaintiff's allegation is unreasonable.

When multiple patent rights have been infringed, it should be regarded that the profit made by the infringer by the act of infringement as referred to in Article 102, paragraph (2) of the Patent Act with regard to the infringement of one patent right is not the entire amount of profits of the infringer. Even if such interpretation is not adopted, at least the rebuttal of the presumption should be allowed according to the number of patent rights infringed and the degree of contribution of the patent rights, as mentioned in 4. (2) below.

4. Regarding grounds for the rebuttal of the presumption

The presumption of the value of damages under Article 102, paragraph (2) of the Patent Act should be rebutted based on the following circumstances.

(1) Sale of competitive products (reduction of the amount according to the market share)

Regarding both the profits for the portion sold by the Defendant and the profits for the portion sold by CJ Indonesia, the value of damages should be reduced according to the market share

The MSG in question is a chemical substance called monosodium glutamate. As the

products do not change depending on their production process, customers are not interested in the production process. Even if the Defendant or CJ Indonesia could not sell the MSG in question, the Plaintiff would not have been able to sell all of the portion that could not be sold by the Defendant or CJ Indonesia, but would only have been able to sell the portion according to the Plaintiff's own market share.

When analysis is made based on the trade statics by the Ministry of Finance (Reference Materials 7 attached to Exhibit Otsu 101, Exhibit Otsu 117), out of the monosodium glutamate imported from Indonesia into Japan, excluding the portion sold by the Defendant, etc., half was produced and sold by the Plaintiff. When also taking imports from other countries into consideration, out of the import volume of monosodium glutamate, excluding the portion sold by the Defendant, etc., approximately ●(omitted)●% was produced and sold by the Plaintiff.

(2) Degree of contribution of the Patent Rights to damages

The Patents only contribute to a limited part of the processes for producing glutamic acid that comprise complicated and diverse steps.

The production of glutamic acid is largely divided into the fermentation phase and the purification phase, and the fermentation phase comprises at least six steps.

Patent 1 relates to a biosynthesis, which is one of the six steps of the fermentation phase, and further, it relates to one of the four reactions of the biosynthesis. Therefore, its contribution to the process for producing glutamic acid is approximately 2% (1/2 × 1/6 × 1/4 = 1/48) at the most.

Patent 2 relates to technological development on efflux of glutamic acid to outside of the bacterial cell, which is one of the six steps of the fermentation phase. Therefore, its contribution to the process for producing glutamic acid is approximately 8.3% (1/2 × 1/6 = 1/12) at the most.

Accordingly, when calculating the damages under Article 102, paragraph (2) of the Patent Act, the abovementioned percentages should be taken into consideration as the contribution rates of the Patents, or the rebuttal of the presumption to the extent of the abovementioned percentages should be allowed.

5. Regarding overlapped application of Article 102, paragraph (3) of the Patent Act for the rebutted part

It should be regarded that, when presumption under Article 102, paragraph (2) of the Patent Act is to be rebutted based on the existence of competitive products, the provisions of paragraph (3) of that Article should not be allowed to be applied in an overlapped manner to the portion to be rebutted.

End

Attachment 11-2 "Allegations on the Value of Damages 2" (Value of Damages under Article 102, paragraph (3) of the Patent Act for the Overall Portion Sold by the Defendant, etc.)

[Plaintiff's allegations]

1. Outline of the allegations

As mentioned in No. 3, 15. [Plaintiff's allegations] (1) of the text, joint tort is found to be established between the Defendant and CJ Indonesia with regard to the overall portion sold by the Defendant, etc. Therefore, the Defendant is liable for tort due to the infringement of the respective Patent Rights with regard to the overall portion sold by the Defendant, etc.

Therefore, the amount equivalent to royalties for the overall portion sold by the Defendant, etc. during the subject period from January 1, 2007 through December 31, 2017 (as mentioned in 2. below, in relation to Patent Right 2, the period on and after August 23, 2013 within that period) will be the value of damages to be compensated by the Defendant pursuant to Article 102, paragraph (3) of the Patent Act.

2. Royalty rates for the Patent Rights

The reasonable royalty rates for the Patent Rights are 30 yen/kg of the sales volume, respectively.

When converted into a rate against the sales amount for the portion sold by the Defendant, 30 yen/kg will be approximately ●(omitted)●%.

According to the Royalty Rate Data Handbook, the average royalty rate for biotechnology is 6.2%. However, as the royalty rate to be determined in a patent infringement lawsuit should be higher than an ordinary royalty rate and given that the inventions claimed in the Patents have high values and significantly contribute to profits, high royalty rates should be determined. In particular, circumstances such as ●(omitted)● should also be taken into consideration when calculating the royalty rates in this case.

3. Amount equivalent to royalties for Patent Right 1

The total sales volume of the portion sold by the Defendant, etc. during the subject period is ●(omitted)● tons, as mentioned in No. 3, 15. [Plaintiff's allegations] (2) of the text, and the amount obtained by multiplying this volume by 30 yen/kg, which comes to ●(omitted)● yen, will be the amount equivalent to royalties for Patent Right 1.

4. Amount equivalent to royalties for Patent Right 2

(1) Sales volumes during the subject period for Patent Right 2

Of the sales volume relating to the portion sold by the Defendant, etc. for 2013, which is ●(omitted)● tons, the portion for the period from August 23, 2013 through the last

day of that year is presumed to be ●(omitted)● tons, as calculated pro rata based on the number of days.

As the sales volumes for 2014 through 2017 are as shown in 2. of Attachment 10 "List of Sales Values/Volumes," the total sales volume relating to the portion sold by the Defendant, etc. during the subject period for Patent Right 2 comes to ●(omitted)● tons.

(2) Amount equivalent to royalties for the subject period for Patent Right 2

The amount equivalent to royalties for Patent Right 2 will be the amount obtained by multiplying the sales volume of ●(omitted)● tons mentioned in (1) above by 30 yen/kg, which comes to ●(omitted)● yen.

5. Relationship between the claims for compensation for damages caused by the infringement of the Patent Rights

During the subject period for Patent Right 2, both Patent Right 1 and Patent Right 2 were infringed, and the amount equivalent to royalties relating to the infringement of Patent Right 1 is the same as the amount mentioned in 4. (2) above, but with regard to the infringement of the Patent Rights during that period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of a total of ●(omitted)● yen.

As damages throughout the subject period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of a total of ●(omitted)● yen as mentioned in 3. above. In this lawsuit, the Plaintiff claims 900 million yen from that amount as partial claims for the damages.

[Defendant's allegations]

1. Outline of the allegations

As mentioned in No. 3, 15. [Defendant's allegations] (1) of the text, the Defendant is not liable for compensation for damages with regard to the portion sold by CJ Indonesia. Therefore, when calculating the value of damages under Article 102, paragraph (3) of the Patent Act, the portion sold by CJ Indonesia should not be taken into consideration.

In addition, as mentioned in No. 3, 15. [Defendant's allegations] (2) of the text, the Plaintiff's allegations concerning the sales volumes for 2007 through 2010, which should serve as the basis for calculation of the amount equivalent to royalties, are unreasonable.

2. Royalty rates for the Patent Rights

The Plaintiff alleges that the royalty rate should be as high as approximately 18.6% of the sales amount for each of the Patent Rights. However, as this rate considerably deviates from the usual rate, the Defendant disputes the Plaintiff's allegations concerning the royalty rates.

[i] The general royalty rate in the relevant technical field and [ii] the inventions claimed in the Patents both relate to processes for producing general-purpose products,

and alternative technologies exist. In addition, [iii] the inventions claimed in the Patents are all used in only a part of the fermentation process in the overall process for producing glutamic acid, and their contribution to profits and their effects are small. In light of these factors, the royalty rates for the Patent Rights should be low, and a rate not more than 1% will be reasonable.

End

Attachment 11-3 "Allegations on the Value of Damages 3" (Value of Damages under Article 102, paragraph (3) of the Patent Act for the Portion Sold by the Defendant and under Paragraph (2) of that Article for the Portion Sold by CJ Indonesia)

[Plaintiff's allegations]

1. Outline of the allegations

As mentioned in No. 3, 15. [Plaintiff's allegations] (1) of the text, joint tort is found to be established between the Defendant and CJ Indonesia at least with regard to the portion sold by CJ Indonesia.

Therefore, from among the portion sold by the Defendant, etc. during the subject period (in relation to Patent Right 2, the period on and after August 23, 2013 within that period), with regard to the portion sold by the Defendant, the amount equivalent to royalties based on Article 102, paragraph (3) of the Patent Act (the royalty rate is as shown in [Plaintiff's allegations] 2. of Attachment 11-2) will be the value of damages to be compensated by the Defendant, and with regard to the portion sold by CJ Indonesia, damages in an amount equivalent to the profits made by the Defendant, etc. under Article 102, paragraph (2) of the Patent Act will be the value of damages to be compensated by the Defendant.

2. Amount equivalent to royalties for the portion sold by the Defendant

(1) Amount equivalent to royalties for Patent Right 1

The sales volume of the portion sold by the Defendant during the subject period is a total of ●(omitted)● tons, according to No. 3, 15. [Plaintiff's allegations] (2) A. and B. (B) of the text, and the amount obtained by multiplying this volume by 30 yen/kg, which comes to ●(omitted)● yen, will be the amount equivalent to royalties for Patent Right 1.

(2) Amount equivalent to royalties for Patent Right 2

Of the sales volume relating to the portion sold by the Defendant for 2013, which is ●(omitted)● tons, the portion for the period from August 23, 2013 through the last day of that year is presumed to be ●(omitted)● tons, as calculated pro rata based on the number of days. As the sales volumes for 2014 through 2017 are as shown in 2. of Attachment 10 "List of Sales Values/Volumes," the total sales volume relating to the portion sold by the Defendant during the subject period for Patent Right 2 comes to ●(omitted)● tons.

The amount equivalent to royalties for Patent Right 2 will be the amount obtained by multiplying this sales volume of ●(omitted)● tons by 30 yen/kg, which comes to ●(omitted)● yen.

3. Profits made by the Defendant, etc. during the subject period for the portion sold by CJ Indonesia

(1) Sales amount

According to No. 3, 15. [Plaintiff's allegations] (2) A. and B. (C) of the text, the total sales amount relating to the portion sold by CJ Indonesia during the subject period is ● (omitted)● yen.

(2) Regarding the expenses borne by the Defendant, etc.

The amounts of expenses to be deducted are calculated by multiplying the amounts of the production cost for the MSG in question, expenses for international transportation, expenses for domestic transportation, and other selling, general, and administrative expenses mentioned in [Plaintiff's allegations] 2. (2) of Attachment 11-1 "Allegations on the Value of Damages 1" by the percentage of the total sales volume of the portion sold by CJ Indonesia in the total sales volume of the portion sold by the Defendant, etc. during the subject period.

The production cost for the MSG in question, expenses for domestic transportation, and other selling, general, and administrative expenses come to a total of ●(omitted)● yen, and the expenses for international transportation come to a total of ●(omitted)● yen.

(3) Profits made by the Defendant, etc.

When the expenses to be deducted mentioned in (2) above are deducted from the sales amount mentioned in (1) above, the profits made by the Defendant, etc. in relation to the portion sold by CJ Indonesia during the subject period come to a total of 3,110,343,000 yen.

4. Profits made by the Defendant, etc. during the subject period for Patent Right 2 (August 23, 2013 through December 31, 2017) for the portion sold by CJ Indonesia

(1) Sales amount

Of the sales amount relating to the portion sold by CJ Indonesia for 2013, which is ●(omitted)● yen, the portion for the period from August 23, 2013 through the last day of that year is presumed to be ●(omitted)● yen, as calculated pro rata based on the number of days. As the sales volumes for 2014 through 2017 are as shown in 1. of Attachment 10 "List of Sales Values/Volumes," the sales amount relating to the portion sold by CJ Indonesia during the subject period for Patent Right 2 comes to ●(omitted)● yen.

(2) Regarding the expenses borne by the Defendant, etc.

When the amounts of expenses to be deducted in relation to the portion sold by CJ Indonesia are calculated in a manner similar to [Plaintiff's allegations] 3. (2) of

Attachment 11-1 "Allegations on the Value of Damages 1," the respective amounts will be as follows:

Production cost for the MSG in question ●(omitted)● yen

Expenses for international transportation ●(omitted)● yen

Expenses for domestic transportation and other selling, general, and administrative expenses ●(omitted)● yen

(3) Profits made by the Defendant, etc.

When the expenses to be deducted mentioned in (2) above are deducted from the sales amount mentioned in (1) above, the profits made by the Defendant, etc. in relation to the portion sold by CJ Indonesia during the subject period for Patent Right 2 come to a total of 1,745,876,000 yen.

5. Relationship between the claims for compensation for damages caused by the infringement of the Patent Rights

The value of damages presumed with regard to the infringement of Patent Right 1 will be the sum of the amount equivalent to royalties for the portion sold by the Defendant mentioned in 2. (1) above and the amount of profits made by the Defendant, etc. in relation to the portion sold by CJ Indonesia mentioned in 3. (3) above, which comes to ●(omitted)● yen.

The value of damages presumed with regard to the infringement of Patent Right 2 will be the sum of the amount equivalent to royalties for the portion sold by the Defendant mentioned in 2. (2) above and the amount of profits made by the Defendant, etc. in relation to the portion sold by CJ Indonesia mentioned in 4. (3) above, which comes to ●(omitted)● yen.

With regard to damages for the subject period for Patent Right 2, during which both of the Patent Rights were infringed, the Plaintiff selectively makes claims based on the Patent Rights within the scope of the abovementioned total value of damages of ●(omitted)● yen.

As damages throughout the subject period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of a total of ●(omitted)● yen as mentioned above. In this lawsuit, the Plaintiff claims 900 million yen from that amount as partial claims for the damages.

6. Regarding grounds for the rebuttal of the presumption with regard to the portion sold by CJ Indonesia

There are no grounds based on which the presumption of damages under Article 102, paragraph (2) of the Patent Act should be rebutted with regard to the portion sold by CJ Indonesia, as mentioned in [Plaintiff's allegations] 5. of Attachment 11-1 "Allegations on

the Value of Damages 1." In addition, supposing that the rebuttal of the presumption of damages were to be allowed based on the existence of competitive products, the value of damages in an amount equivalent to royalties under Article 102, paragraph (3) of the Patent Act should be recognized for the rebutted part, as mentioned in [Plaintiff's allegations] 6. of that Attachment.

[Defendant's allegations]

As mentioned in No. 3, 15. [Defendant's allegations] (1) of the text, the Defendant is not liable for compensation for damages with regard to the portion sold by CJ Indonesia. Therefore, when calculating the value of damages under Article 102, paragraph (2) of the Patent Act, the portion sold by CJ Indonesia should not be taken into consideration.

In addition, the Defendant makes allegations concerning the value of damages under Article 102, paragraph (3) of the Patent Act with regard to the portion sold by the Defendant as described in [Defendant's allegations] of Attachment 11-2 "Allegations on the Value of Damages 2", and makes allegations concerning the value of damages under paragraph (2) of that Article with regard to the portion sold by CJ Indonesia as described in [Defendant's allegations] of Attachment 11-1 "Allegations on the Value of Damages 1."

End

Attachment 11-4 "Allegations on the Value of Damages 4" (Value of Damages under Article 102, Paragraph (3) of the Patent Act for the Portion Sold by the Defendant and under Paragraph (2) of That Article for the Defendant's Commissions for the Portion Sold by CJ Indonesia)

[Plaintiff's allegations]

1. Outline of the allegations

Irrespective of whether joint tort may be established between the Defendant and CJ Indonesia for the portion sold by CJ Indonesia, from among the portion sold by the Defendant, etc. during the subject period (in relation to Patent Right 2, the period on and after August 23, 2013 within that period), with regard to the portion sold by the Defendant, compensation for damages in an amount equivalent to royalties based on Article 102, paragraph (3) of the Patent Act may be claimed, and with regard to the portion sold by CJ Indonesia, compensation for damages in an amount equivalent to commissions, which are profits made by the Defendant in relation to that portion, may be claimed as the value of damages under Article 102, paragraph (2) of the Patent Act.

2. Amount equivalent to royalties for the portion sold by the Defendant

The same as in [Plaintiff's allegations] 2 of Attachment 11-3.

3. Profits made by the Defendant in an amount equivalent to commissions relating to the portion sold by CJ Indonesia

(1) Profits made by the Defendant (the amount of commissions) during the subject period

According to material disclosed by the Defendant (Reference Materials 3 attached to Exhibit Otsu 101), the Defendant has received payment of commissions (fees) from CJ Indonesia for the portion sold by CJ Indonesia, and they constitute profits (Article 102, paragraph (2) of the Patent Act) made by the Defendant from the act of infringement, which is an act of offering to transfer the products, conducted by the Defendant with regard to the portion sold by CJ Indonesia.

The total amount of commissions for 2011 through 2017 is ●(omitted)● yen, and it is expected that the Defendant also received payment of commissions of at least the same amount as that for 2011, which is ●(omitted)● yen, in each year from 2007 through 2010. Therefore, the total amount of commissions paid during the subject period is ●(omitted)● yen, and this amount is presumed to be damages sustained by the Plaintiff, pursuant to Article 102, paragraph (2) of the Patent Act.

(2) Profits made by the Defendant (the amount of commissions) during the subject period for Patent Right 2 (August 23, 2013 through December 31, 2017)

Of the commissions for 2013, which is ●(omitted)● yen, the portion for the period

from August 23, 2013 through the last day of that year is presumed to be ●(omitted)● yen, as calculated pro rata based on the number of days.

When the commissions for 2014 through 2017 are added up, the total amount of commissions paid to the Defendant during the subject period for Patent Right 2 comes to ●(omitted)● yen.

4. Relationship between the claims for compensation for damages caused by the infringement of the Patent Rights

The value of damages presumed with regard to the infringement of Patent Right 1 will be the sum of the amount equivalent to royalties for the portion sold by the Defendant ([Plaintiff's allegations] 2. (1) of Attachment 11-3 "Allegations on the Value of Damages 3") and the amount of commissions mentioned in 3. (1) above, relating to the subject period, which comes to ●(omitted)● yen.

The value of damages presumed with regard to the infringement of Patent Right 2 will be the sum of the amount equivalent to royalties for the portion sold by the Defendant ([Plaintiff's allegations] 2. (2) of Attachment 11-3 "Allegations on the Value of Damages 3") and the amount of commissions mentioned in 3. (2) above, relating to the subject period for Patent Right 2, which comes to ●(omitted)● yen.

With regard to damages for the subject period for Patent Right 2, during which both of the Patent Rights were infringed, the Plaintiff selectively makes claims based on the Patent Rights within the scope of the abovementioned total value of damages of ●(omitted)● yen.

As damages throughout the subject period, the Plaintiff selectively makes claims based on the Patent Rights within the scope of a total of ●(omitted)● yen as mentioned above. In this lawsuit, the Plaintiff claims 900 million yen from that amount as partial claims for the damages.

5. Regarding grounds for the rebuttal of the presumption with regard to the portion sold by CJ Indonesia

There are no grounds based on which the presumption of damages under Article 102, paragraph (2) of the Patent Act should be rebutted with regard to the portion sold by CJ Indonesia, as mentioned in [Plaintiff's allegations] 5. of Attachment 11-1 "Allegations on the Value of Damages 1." In addition, supposing that the rebuttal of the presumption of damages were to be allowed based on the existence of competitive products, the value of damages in an amount equivalent to royalties under Article 102, paragraph (3) of the Patent Act should be recognized for the rebutted part, as mentioned in [Plaintiff's allegations] 6. of that Attachment.

[Defendant's allegations]

1. Regarding the value of damages under Article 102, paragraph (3) of the Patent Act with regard to the portion sold by the Defendant

The Defendant makes allegations as described in [Defendant's allegations] of Attachment 11-2 "Allegations on the Value of Damages 2."

2. Regarding allegations based on Article 102, paragraph (2) of the Patent Act treating commissions as profits made by the Defendant in relation to the portion sold by CJ Indonesia

Commissions are profits which the Defendant can independently gain based on a commission contract concluded with CJ Indonesia. On the other hand, the Plaintiff does not conduct business relating to commissions. Therefore, the Plaintiff and the Defendant are not in a competitive relationship in business relating to commissions, and the Defendant's obtainment of commissions does not deprive the Plaintiff of the opportunity to obtain commissions. Accordingly, commissions should not be taken into consideration as the profits made by the Defendant under Article 102, paragraph (2) of the Patent Act.

Supposing that commissions were to be taken into consideration as profits made by the Defendant, the amount of commissions received in each year from 2007 through 2010 is not the same as the amount received in 2011. The commissions for 2007 through 2010 should also be estimated in a manner similar to that for the sales amounts, by a formula similar to that mentioned in No. 3, 15. [Defendant's allegations] (2) B. of the text, which will come to a total of ●(omitted)● yen. In addition, the related expenses (the selling, general, and administrative expenses for the portion sold by CJ Indonesia in [Defendant's allegations] 2. (1) of Attachment 11-1 "Allegations on the Value of Damages 1") should also be taken into consideration in relation to commissions.

Moreover, in a manner similar to [Defendant's allegations] 4. (1) of Attachment 11-1 "Allegations on the Value of Damages 1," the amount of profits made by the Defendant through commissions should also be reduced (rebutted) based on the Plaintiff's market share, and overlapped application of Article 102, paragraph (3) of the Patent Act should not be allowed for the rebutted part, as in [Defendant's allegations] 5. of that Attachment.

End

Attachment 12 "Statements in Description 1"

The detailed explanation of the invention in Description 1 concerning Corrected Invention 1 contains the following statements:

1. Technical Field

[0001]

The present invention relates to a method of constructing a mutant strain capable of producing amino acids in a high yield, and a method of producing L-amino acids by the fermentation with the mutant.

2. Background Art

[0002]

Methods of constructing mutant strains usable for the production of amino acids by the fermentation can be roughly classified into two methods. One of them comprises introducing random mutations into DNA with a chemical mutagen, and the other comprises the genetic recombination. In the latter method, a strain having an improved capacity of producing an intended substance can be developed by enhancing a gene on a metabolic pathway relating to the biosynthesis of an intended substance, or by weakening a gene of an enzyme relating to the destruction. In this connection, for enhancing an intended gene, a plasmid capable of autonomously replicating independently from the chromosome in a cell has been mainly used.

However, the method of enhancing the intended gene with a plasmid has problems. In particular, the degree of enrichment of the intended gene is variable depending on the number of copies of the plasmid itself. Therefore, for some kinds of intended genes, the copies are often too many in number and, as a result, the expression becomes excessive, the growth is seriously inhibited or the capacity of producing the intended substance is lowered. In such a case, although the degree of the enhancement of the intended gene can be lowered by using a plasmid of a small number of the copies, the variety of the plasmid is limited in many cases, and the intended control of the expression level of the intended gene is impossible.

[0003]

Another problem is that since the replication of the plasmid is often unstable, the plasmid is eliminated.

3. Problem to be Solved by the Invention

[0006]

The object of the present invention is to provide a method of constructing a mutant

capable of suitably enhancing or controlling the expression of an intended gene without using a plasmid and also capable of producing amino acids in a high yield, by gene recombination or mutation.

Another object of the present invention is to provide a promoter for GDH capable of imparting a capability of producing glutamic acid in a high yield to a *Corynebacterium* strain without seriously increasing the amount of by-produced aspartic acid and alanine.

Still another object of the present invention is to provide a GDH gene having a sequence of the above-described promoter for GDH.

A further object of the present invention is to provide a *Corynebacterium* strain having the above-described gene and capable of producing L-glutamic acid.

A further object of the present invention is to provide a method of producing amino acids by fermentation wherein amino acid-producing microorganism thus constructed is used.

A further object of the present invention is to provide a fermentation method of producing glutamic acid at a low cost by increasing the yield of glutamic acid by using a glutamic acid-producing coryneform bacterium.

4. Means for Solving the Problem

[0007]

The present invention has been completed on the basis of a finding that the above-described problems can be efficiently solved by variously modifying the promoter of amino acid-biosynthesizing genes on a chromosome to control the amount of the expression of the intended genes. Particularly, the invention has been completed on the basis of a finding that the above-described problem can be efficiently solved by introducing a specific mutation into -35 region or -10 region which is a specific region of the promoter.

Namely, the present invention provides a method of producing coryneform bacteria having an improved amino acid- or nucleic acid-productivity, which comprises the steps of introducing a mutation in a promoter sequence of amino acid- or nucleic acid-biosynthesizing genes on a chromosome of a coryneform bacterium to make it close to a consensus sequence or introducing a change in a promoter sequence of amino acid- or nucleic acid-biosynthesizing genes on a chromosome of a coryneform bacterium by gene recombination to make it close to a consensus sequence, to obtain mutants of the coryneform amino acid- or nucleic acid-producing microorganism, culturing the mutants, and selecting a mutant capable of producing the intended amino acid or nucleic acid in a large amount.

[0008]

The present invention also provides a promoter for glutamate dehydrogenase (GDH)-producing gene, which has the sequence of (i) at least one DNA sequence selected from the group consisting of CGGTCA, TTGTCA, TTGACA and TTGCCA in -35 region, (ii) TATAAT sequence or the same TATAAT sequence but in which the base of ATAAT is replaced with another base in -10 region, or (iii) a combination of (i) and (ii), wherein the sequence does not inhibit the promoter function.

The present invention also provides a glutamate dehydrogenase-producing gene having the above-described promoter.

The present invention also provides a coryneform L-glutamate-producing microorganism having the above-described gene.

[0009]

The present invention also provides a process for producing an amino acid by the fermentation, which comprises the steps of culturing a coryneform bacterium constructed by the above-described method and having an improved amino acid-producing capacity in a medium to form and also to accumulate the intended amino acid in the medium, and collecting the amino acid from the medium.

The present invention also provides a process for producing L-glutamic acid by the fermentation, which comprises the steps of culturing a coryneform L-glutamic acid-producing microorganism resistant to 4-fluoroglutamic acid in a liquid medium to form and also to accumulate L-glutamic acid in the medium, and collecting L-glutamic acid from the medium.

5. Best Mode for Carrying Out the Invention

[0010]

The term "coryneform glutamic acid producing microorganism" as used herein includes also bacteria which were classified to be the genus *Brevibacterium* before but now integrated into the genus *Corynebacterium* [Int. J. Syst. Bacteriol., 41, 255 (1981)] and also bacteria of the genus *Brevibacterium* which are very close to those of the genus *Corynebacterium*. Therefore, the mutants used in the present invention can be derived from the coryneform glutamic acid-producing bacteria of the genus *Brevibacterium* or *Corynebacterium* shown below. Bacteria of the genus *Corynebacterium* and those of the genus *Brevibacterium* will be collectively referred to as "coryneform bacteria" so far as they do not concern the glutamic acid productivity.

[0011]

... *Corynebacterium glutamicum* ATCC13032

... *Brevibacterium lactofermentum* ATCC13869...

[0012]

The amino acids to be produced are not particularly limited so far as the genes concerning the biosynthesis and promoters thereof have been elucidated. Examples of effective enzymes concerning the biosynthesis include GDH, citrate synthase (CS), isocitrate dehydrogenase (ICDH), pyruvate dehydrogenase (PDH) and aconitase (ACO) for glutamic acid fermentation. ...

[0015]

In the present invention, a mutant of a coryneform amino acid-producing bacterium is obtained by, introducing a mutation in a promoter sequence of desired amino acid-biosynthesizing genes on a chromosome of a coryneform amino acid-producing bacterium, such as the above-described promoter sequence for GDH, to make it close to a consensus sequence with a chemical or by introducing the mutation by the genetic recombination to obtain a mutant of the coryneform amino acid-producing microorganism.

The term "consensus sequence" is a sequence which appears most frequently in various promoter sequences. Such consensus sequences include, for example, those of *E. coli* and *Bacillus subtilis*. The consensus sequence of *E. coli* is described in Diane K. Hawley and William R. McClure Nuc. Acid. Res. 11:2237-2255 (1983), and that of *B. subtilis* is described in Charles et al. Mol. Gen. Genet 186:339-346 (1982).

The mutation may be caused in either only one promoter sequence such as that for GDH or two or more promoter sequences such as those for GDH, citrate synthase (citrate-synthesizing enzyme) (CS) and isocitrate dehydronenase (isocitrate-synthesizing enzyme) (ICDH).

In the present invention, the mutant thus obtained is cultured to obtain the mutant capable of producing a large amount of an intended amino acid.

[0016]

It was already elucidated that in the fermentation of glutamic acid, GDH derived from a coryneform glutamate-producing microorganism has its own promoter sequence in upstream region thereof [Sahm et al., Molecular Microbiology (1992), 6, 317-326].

For example, the promoter for GDH of the present invention, GDH gene having the promoter sequence for GDH and L-glutamate-producing *Corynebacterium* strain having this gene can be obtained by, for example, the following methods:

Namely, the strain is subjected to a mutagenesis treatment such as the irradiation with UV, X-rays or radiation, or treatment with a mutagen to obtain a strain resistant to 4-fluoroglutamic acid on an agar plate culture medium containing 4-fluoroglutamic acid. Namely, the mutagenized cells are spread on an agar plate culture medium containing 4-fluoroglutamic acid in such a concentration that it inhibits the growth of the parent, and

the mutant thus grown is separated.

Further, the promoter sequence of GDH genes can be replaced with variously modified sequences by site directed mutagenesis, and the relationship between the respective sequences and GDH activity is examined so as to select the ones having a high L-glutamate-productivity.

[0017]

It is particularly preferred in the present invention that the DNA sequence in -35 region of the promoter for GDH-producing gene is at least one DNA sequence selected from the group consisting of CGGTCA, TTGTCA, TTGACA and TTGCCA and/or the DNA sequence in -10 region of the promoter is TATAAT, or the bases of ATAAT in TATAAT sequence in -10 region is replaced with another base, while they do not inhibit the promoter function. The reason why the strain in which the bases of ATAAT in TATAAT sequence in -10 region is replaced with another base and the promoter function is not inhibited can be selected is as follows: Because a remarkable increase in the specific activity of GDH was observed by merely replacing the first "C" of CATAAT with "T" in wild type -10 sequence (refer to p6-4 in Table 1), it was considered that such a replacement with another base is possible.

The promoter sequence of GDH gene is described in, for example, the above-described Sahm et al., Molecular Microbiology (1992), 6, 317-326. It is described therein as Seq ID No. 1. The sequence of GDH gene itself is also described in Sahm et al., Molecular Microbiology (1992), 6, 317-326 to be Seq ID No. 1.

Similarly, the mutation can be introduced in the promoter for citrate-synthesizing enzyme (CS) or isocitrate dehydrogenase (ICDH).

Thus, the promoters for GDH are those having at least one DNA sequence selected from the group consisting of CGGTCA, TTGTCA, TTGACA and TTGCCA in -35 region and/or TATAAT sequence or the TATAAT sequence but in which the base of ATAAT is replaced with another base in -10 region, wherein they do not inhibit the promoter function. Genes for producing glutamate dehydrogenase, which have the above-described promoter, are also provided.

[0018]

The promoters for CS are those having TTGACA sequence in -35 region and/or TATAAT sequence in -10 region, which do not inhibit the promoter function. CS genes having the above-described promoter are also provided. ...

The present invention also provides coryneform L-glutamate-producing bacterium having the above-described genes.

[0020]

The coryneform bacteria usually produce L-glutamic acid under reduced biotin condition. Therefore, the amount of biotin in the medium is restricted or a substance inhibiting the effect of biotin such as a surfactant or penicillin is added.

The fermentation is preferably conducted by shaking the culture or agitating the culture with aeration while the pH of the culture liquid is kept in the range of 5 to 9 for 2 to 7 days. The pH is preferably controlled with urea, calcium carbonate, gaseous ammonia, ammonia water or the like. The culture temperature is preferably 24 to 37°C.

L-glutamic acid thus produced and accumulated in the culture liquid is collected by an ordinary method such as ion-exchange resin method or crystallization method. Specifically, L-glutamic acid is separated by the adsorption on an anion-exchange resin or by the neutralization crystallization.

According to the present invention, the intended amino acid can be obtained in a high yield by introducing a mutation into a promoter region of amino acid-biosynthesizing genes of a coryneform amino acid-producing bacterium to control the expression of the intended genes. In addition, since any elimination of the intended gene does not occur in the bacteria according to the present invention, contrary to the cases using plasmid, the intended amino acid can be stably obtained in a high yield. Thus, the industrial merit of the invention is great.

[0021]

The present invention provides various promoters, particularly, promoters for GDH, capable of imparting a power of producing amino acids, particularly glutamic acid, in a high yield to *Corynebacterium* strains without increasing the amount of by-produced aspartic acid and alanine.

In the present invention, a coryneform L-glutamate-producing bacterium is mutagenized, a strain in which the mutation is introduced in a promoter region of GDH gene and which is resistant to 4-fluoroglutamic acid is collected, and the strain is cultured to obtain glutamic acid in a high yield. Thus, the present invention is industrially very advantageous.

6. Working Examples

(1) Example 1　Production of Mutant GDH Promoter

[0022]

... A mutant GDH promoter was prepared by site-directed mutagenesis method as follows:

(1) Preparation of GDH Genes Having Various Mutant Promoters:

The wild type sequence in -35 region and -10 region of a promoter of GDH gene of a coryneform bacteria is shown in sequence 1. The promoter sequence of wild type has already been reported [Molecular Microbiology (1992), 6, 317-326].

The method of preparing a plasmid carrying GDH gene having a mutant promoter is as follows:

As shown in FIG. 1, a chromosomal gene of a wild type strain of a coryneform bacterium ATCC13869 prepared with "Bacterial Genome DNA purification kit" (Advanced Genetic Technologies Corp.) was used as the template for PCR. The gene amplification was conducted by PCR using upstream and downstream sequences of GDH gene. Both ends were blunt-ended. The product thus obtained was inserted in SmaI site of plasmid pHSG399 (a product of Takara Shuzo Co., Ltd.). Then a replication origin taken from plasmid pSAK4 having the replication origin capable of replicating in a coryneform bacterium was introduced into Sal I site of the plasmid to obtain plasmid pGDH. By this method, GDH genes having each above-described promoter sequence can be obtained by using a primer having each of the sequence of Seq ID No. 1 to Seq ID No. 6 shown in the Sequence Listing as the upstream primer for GDH gene, respectively. It was confirmed by sequencing the PCR amplified fragment that any mutation, other than the introduced mutation in the promoter sequence, did not occur in the amplified fragment. pSAK4 is constructed as follows: previously obtained plasmid pHK4 (Japanese Unexamined Patent Application Publication No. 1993-7491) having an autonomous replication origin derived from plasmid pHM1519 [Agric. Biol. Chem., 48, 2901-2903 (1984)] which is capable of autonomously replicating in *Corynebacterium* microorganism, is digested with restriction enzymes BamHI and KpnI to obtain a DNA fragment having the replication origin. Then the fragment thus obtained is blunt-ended with DNA-Blunting Kit (Blunting kit of Takara Shuzo Co., Ltd.). After the ligation with Sal I linker (a product of Takara Shuzo Co., Ltd.), the product thus obtained was inserted into Sal I site of pHSG299 to obtain plasmid pSAK4.

[0023]

(2) Comparison of the Degrees of Expression of GDH Having Each Promoter Sequence:

Each plasmid prepared as described above was introduced into wild type strain of coryneform bacterium ATCC13869 by electroporation method (refer to Japanese Unexamined Patent Application Publication No. 1990-207791). For comparing the degrees of expression of GDH for these strains, the specific activity of GDH was determined by the above-described method of Sahm et al. The results are shown in Table 1.

[0024]

Table 1

Strain	Promoter sequence		Specific activity of GDH	Relative value
	-35	-10		

ATCC 13869		TGGTCA	CATAAT	7.7	0.1
	/pGDH	TGGTCA	CATAAT	82.7	1.0
	/p6-2	CGGTCA	CATAAT	33.1	0.4
	/p6-4	TGGTCA	TATAAT	225.9	2.7
	/p6-3	TTGACA	TATAAT	327.2	4.0
	/p6-7	TTGCCA	TATAAT	407.0	4.9
	/p6-8	TTGTCA	TATAAT	401.3	4.9

[0025]

ATCC 13869/p6-2 through ATCC 13869/p6-8 corresponded to the sequences of Seq ID No. 2 through Seq ID No. 6, respectively. These sequences were the same as the sequence of Seq ID No. 1 (wild type) except that the underlined parts were changed as follows:

Seq ID No. 1	5'-TTAATTCTTTGTGGTCATATCTGCGACACTGC	CATAATTTGAACGT- 3'
Seq ID No. 2	CGGTCA	CATAAT
Seq ID No. 3	TGGTCA	TATAAT
Seq ID No. 4	TTGACA	TATAAT
Seq ID No. 5	TTGCCA	TATAAT
Seq ID No. 6	TTGTCA	TATAAT

These were those of synthetic linear doubled stranded DNA.

(2) Example 2 Preparation of Mutant Strains

[0026]

... (1) Preparation of Mutant Strains Resistant to 4-Fluoroglutamic Acid:

AJ13029 is a mutant strain producing glutamic acid and disclosed in WO96/06180. Although it does not produce glutamic acid at a culture temperature of 31.5°C., it produces glutamic acid even in the absence of a biotin-inhibitor when the culture temperature is shifted to 37°C. In this Example, *Brevibacterium lactofermentum* AJ13029 strain was used as the parent strain for preparing the mutant strains. As a matter of course, any of glutamic acid-producing strains other than AJ13029 can be used as a parent strain for preparing mutant strains resistant to 4-fluoroglutamic acid.

AJ13029 was cultured on a CM2B agar medium (...) at 31.5°C. for 24 hours to obtain the bacterial cells. The cells were treated with 250 μg/ml aqueous solution of N-methyl-N'-nitro-N-nitrosoguanidine at 30°C. for 30 minutes. Then a suspension of the cells having a survival rate of 1% was spread on agar plates culture medium (...) containing 4-fluoroglutamic acid (4FG). Colonies were formed after incubating the plate at 31.5°C. for 20 to 30 hours. In this experiment, a slant medium containing 1 mg/ml of 4FG was prepared at first, and then a layer of the same medium without 4FG was formed thereon

horizontally. Thus, 4FG concentration gradient was obtained on the surface of the agar medium. When the plate was inoculated with the mutant cells obtained as described, a boundary line was formed at a border of the growing limit of the strain. Bacterial strains which formed colonies in an area containing 4FG of a concentration higher than that of the boundary line were taken. Thus, about 50 strains resistant to 4FG were obtained from about 10,000 mutagenized cells.

[0029]

(2) Confirmation of Capability of L-Glutamic Acid-Production of 4FG-Resistant Mutant Strains:

The capability of glutamic acid-production of about 50 mutant strains obtained in above (1) and parent AJ13029 strain were confirmed as described below.

AJ13029 and mutant strains were each ... After the completion of the culture, the culture was examined with a Biotic Analyzer (a product of Asahi Chemical Industry Co., Ltd.) to determine whether L-glutamic acid was produced or not. It was thus found that when the 50 strains were cultured, two strains having a yield of glutamic acid higher than that obtained from the parent strains and a high GDH activity were separated (strain A and strain B). GDH activity of each of them was determined to find that the specific GDH activity of both of them was increased (Table 5). The GDH activity was determined by the method of E. R. Bormann et al. [Molecular Microbiol., 6, 317-326 (1996)]. By sequencing the GDH genes, it was identified that the mutation points were found only in the promoter region of GDH (Table 6).

[0031]

Table 5 Glutamic acid formation and GDH activity of mutant strains

Strain	Glu (g/dl)	GDH specific activity	Relative value
AJ13029	2.6	7.7	1.0
FGR1	2.9	23.1	3.0
FGR2	3.0	25.9	3.4

[0032]

Table 6 DNA sequences in GDH promoter region of mutant strains

Strain	GDH promoter sequence		
	-35		-10
AJ13029	TGGTCA	TTCTGTGCGACACTGC	CATAAT
FGR1	TGGTCA	TTCTGTGCGACACTGC	TATAAT
FGR2	TTGTCA	T-CTGTGCGACACTGC	TATAAT

(3) Example 3 Introduction of Mutation into CS Gene Promoter Region of Coryneform Glutamate-Producing Bacterium:

[0033]

... In this Example, a strain having an enhanced promoter for the genes which code glutamate dehydrogenase (GDH) and citrate-synthesizing enzyme (CS) was produced.

(1) Cloning of gltA Gene:

The sequence of gltA gene of a coryneform bacterium, which codes citrate-synthesizing enzyme, has already been elucidated [Microbial. 140, 1817-1828 (1994)]. On the basis of this sequence, primers shown in Seq ID No. 7 and Seq ID No. 8 were synthesized. On the other hand, chromosomal DNA from *Brevibacterium lactofermentum* ATCC13869 was prepared using Bacterial Genome DNA Purification Kit (Advanced Genetic Technologies Corp.). Sterilized water was added to a mixture of 0.5 μg of the chromosomal DNA, 10 pmol of each of the oligonucleotides, 8 μl of dNTP mixture (2.5 mM each), 5 μl of 10×La Taq Buffer (Takara Shuzo Co., Ltd.) and 2 U of LA Taq (Takara Shuzo Co., Ltd.) to obtain 50 μl of PCR reaction cocktail. The reaction cocktail was subjected to PCR. The PCR conditions were 30 cycles of denaturation at 94°C. for 30 seconds, annealing at 55°C. for 15 seconds and extension at 72°C. for 3 minutes using Thermal Cycler TP240 (Takara Shuzo Co., Ltd.) to amplify about 3 Kbp of DNA fragments containing gltA gene and promoter thereof. The amplified fragments thus obtained were purified with SUPREC02 (Takara Shuzo Co., Ltd.) and then blunt-ended. The blunting was conducted with Blunting Kit of Takara Shuzo Co., Ltd. The blunt-ended fragment was mixed with pHSG399 (Takara Shuzo Co., Ltd.) completely digested with SmaI to conduct the ligation. The ligation reaction was conducted with DNA Ligation Kit ver 2 (Takara Shuzo Co., Ltd.). After the completion of the ligation, the transformation was conducted with competent cells of *E. coli* JM109 (Takara Shuzo Co., Ltd.). The cells were spread on L medium plates (comprising 10 g/l of bactotryptone, 5 g/l of bactoycast extract, 5 g/l of NaCl and 15 g/l of 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-indoyl-β-D-galactoside) and 40 μg/ml of chloramphenicol. After culturing them overnight, white colonies were taken to obtain the transformed strains after single colony isolation.

From the transformed strains, plasmids were prepared by the alkali method (*Seibutsu Kogaku Jikken-sho* edited by Nippon Seibutsu Kogaku-kai and published by Baifukan, p. 105, 1992). Restriction enzyme maps were prepared, and the plasmid which has the same restriction map as the map shown in FIG. 2 was named "pHSG399CS".

[0034]

(2) Introduction of Mutations into gltA Promoter:

Mutan-Super Express Km (Takara Shuzo Co., Ltd.) was used for the introduction of mutation into gltA promoter region.

[0035]

... The plasmids in which gltA promoter region was replaced with the sequence shown in Table 7 were named pKF19CS1, pKF19CS2 and pKF19CS4, respectively.

[0036]

Table 7

	-35 region	-10 region
pKF19CS	ATGGCT	TATAGC
pKF19CS1	ATGGCT	TATAAC
pKF19CS2	ATGGCT	TATAAT
pKF19CS4	TTGACA	TATAAT

[0041]

(5) Introduction of Mutant gltA Gene into Temperature-Sensitive Plasmid:

For integrating mutant gltA promoter sequences into a chromosome, a method is known wherein a plasmid of which replication in a coryneform bacterium is temperature sensitive is used (Japanese Unexamined Patent Application Publication No. 1993-7491). ... From the transformants, plasmids were prepared. Temperature-sensitive shuttle vectors containing gltA gene were named pSFKTC1, pSFKTC2 and pSFKTC4, respectively.

[0042]

(6) Introduction of Mutant gltA Promoter into Chromosome:

pSFKTC1, pSFKTC2 and pSFKTC4 were each introduced into *Brevibacterium lactofermentum* FGR2 strain by electrical pulse method. ... It was thus proved that in this strain, mutant gltA gene derived from the temperature-sensitive plasmid was integrated near gltA gene in the host chromosome by homologous recombination. Strains derived from pSFKTC1, 2 and 4 were named BLCS11, BLCS12 and BLCS14, respectively.

[0043]

(7) Preparation of Substituted gltA Promoters:

First, kanamycin-sensitive strains were obtained from the strains BLCS11, BLCS12 and BLCS14 having mutant gltA gene integrated therein by homologous recombination. ...

The chromosome was extracted from the kanamycin-sensitive strain, and PCR was conducted with primers having the sequence shown in Seq ID No. 7 and Seq ID No.8 to prepare gltA gene fragments. The amplified fragments thus obtained were purified with SUPREC02 (Takara Shuzo Co., Ltd.) and then subjected to the sequencing reaction using a primer of Seq ID No. 13 to determine the sequence in the promoter region thereof. As a result, the strain having the same promoter sequence as that of pKF19CS1 in Table 7 was named GB01, the strain having the same promoter sequence as that of pKF19CS2

was named GB02 and the strain having the same promoter sequence as that of pKF19CS4 was named GB03. It was indicated that in these strains, the gltA gene of wild type originally located on the chromosome was excised together with the vector plasmid while the mutant gltA gene introduced by the plasmid remained on the chromosome when the plasmid and duplicated gltA gene were excised from the chromosome.

[0044]

(8) Determination of Activity of Citrate Synthase of Mutant gltA Promoter Strains:

The activities of the citrate synthase were determined by treating FGR2, GB01, GB02, GB03 and FGR2/pSFKC strains obtained in step (7) ... The results are shown in Table 10. It was confirmed that the citrate synthase activity of the substituted gltA promoter strain was higher than that of the parent strains thereof.

[0045]

Table 10

Strain	dABS/min/mg	Relative activity
FGR2	7.9	1.0
GB01	9.5	1.2
GB02	15.0	1.9
GB03	31.6	4.0
FGR2/pSFKC	61.6	7.8

[0046]

(9) Results of Culture of Substituted gltA Promoter Strains:

Each of the strains obtained in above-described step (7) was ... The culture was terminated ... and the quantity of L-glutamic acid formed and accumulated in the culture liquid was determined.

As a result, the larger improvement in the yield of L-glutamic acid was confirmed when each of the strains GB02 and GB03 rather than GB01 and FGR2/pSFKC was used as shown in Table 12. From these facts, it was found that good results were obtained by introducing the mutation into the gltA promoter to increase the CS activity to 2 to 4 times for the improvement in the yield of glutamic acid produced by those strains.

[0048]

Table 12

Strain	L-glutamic acid (g/l)
FGR2	8.9
GB01	9.1
GB02	9.4
GB03	9.4

(4) Example 7 Introduction of Mutation into GDH Gene Promoter Region of Coryneform Glutamate-Producing Bacterium

[0087]

... (1) Construction of Mutant gdh Plasmids:

Plasmids having GDH promoter sequence of FGR1 strain and FGR2 strain described in Example 2 were constructed by site directed mutagenesis. For obtaining GDH promoter sequence of FGR1 strain, PCR was conducted by using ... as the primers with chromosomal DNA of ATCC13869 as the template. Further, PCR was conducted by using ... as the primers with a mixture of these PCR products as the template. The PCR product thus obtained was inserted into SmaI site of pSFKT2 (Japanese Patent Application No. 1999-69896) to construct pSFKTG11. To obtain GDH promoter sequence of FGR2 strain, PCR was conducted by using ... as the primers and chromosomal DNA of ATCC13869 as the template; and on the other hand, PCR was conducted by using ... as the primers and chromosomal DNA of ATCC13869 as the template. Further, PCR was conducted by using ... as the primers and a mixture of these PCR products as the template. The PCR product thus obtained was inserted into SmaI site of pSFKT2 (Japanese Patent Application No. 1999-69896) to construct pSFKTG07. The DNA sequences of the fragments inserted into SmaI sites of pSFKTG11 and pSFKTG07 were determined to confirm that no mutation was introduced into other regions than the promoter region of GDH.

[0088]

(2) Construction of gdh Promoter Modified Strains:

Then, pSFKTG11 and pSFKTG07 were introduced into AJ13029 strain by electrical pulse method, and transformants which grew on CM2B plates containing 25 μg/ml of kanamycin at 25°C. were selected. The transformants were cultured at 34°C. to select strains which were resistant to kanamycin at 34°C. The fact that a strain is resistant to kanamycin at 34°C. indicates that pSFKTG11 or pSFKTG07 was thus integrated on the chromosome of AJ13029 strain. Kanamycin-sensitive strains were obtained from the strains in which the plasmid was integrated on the chromosome. The GDH promoter sequences of these strains were determined. The strains having the same gdh promoter sequence as those of pSFKTG11 and pSFKTG07 were named GA01 and GA02, respectively.

(3) Confirmation of L-Glutamic Acid-Productivity of gdh Promoter Modified Strains:

The glutamic acid productivities of strains GA01 and GA02 and the parent strain AJ13029 were confirmed in the same manner as that of Example 2 (2) given above. As a

result, a remarkable improvement in the accumulation of glutamic acid was recognized in GA01 and GA02 as shown in Table 23.

[0089]

Table 23

Strain	Glu (g/dl)	Specific activity of GDH	Relative value
AJ13029	2.6	7.7	1.0
GA01	3.0	22.3	2.9
GA02	2.9	27.0	3.5

[0090]

(4) Construction of Self-Cloning Type gdh Plasmid:

First, self-cloning vector pAJ220 was constructed. ... This plasmid could autonomously replicate in a coryneform bacterium, and it could afford trimethoprim resistance to the host.

PCR reaction was conducted by using ... as the primers and chromosomal DNA of wild-type coryneform bacterium strain ATCC13869 as the template. The gdh gene fragment thus obtained was inserted in BalI site of pAJ220 to construct pAJ220G. ... PAJ220G and pGDH were introduced into ATCC13869 strain by electrical pulse method. GDH activities of the strains thus constructed were determined by the method stated in above-described step (1). As a result, GDH activity of the strain into which pAJ220G had been introduced was about 1.5 times as high as that of the strain into which dGDH had been introduced as shown in Table 24.

[0091]

Table 24

Strain	Specific activity of GDH	Relative value
ATTC13869	7.7	1.0
ATTC13869/pGDH	82.7	10.7
ATTC13869/pAJ220G	120.1	15.6

[0092]

(5) Investigations on Influence of gdh Activity on the Yield and By-Produced Asp:

pGDH and pAJ220G were introduced into AJ13029 by electrical pulse method. Each of these strains and those obtained in above-described step (2) was ... The culture was terminated ... and the quantity of L-glutamic acid produced and accumulated in the culture liquid were determined (Table 26). The GDH activity for obtaining the highest yield was about 3-times as high. When GDH activity was further elevated, the degree of the improvement in the yield was reduced. When the GDH activity was elevated to about 16-times, the yield was rather reduced. Amino acids produced as by-products were analyzed

with Hitachi Amino Acid Analyzer L-8500 to find that as GDH activity was elevated, the amount of accumulated aspartic acid and alanine was increased. These results proved the following facts: For increasing the yield of glutamic acid, it is necessary to suitably increase GDH activity so as not to cause a remarkable increase in the amount of aspartic acid and alanine. One of the effective methods therefor comprises the introduction of various mutations into gdh promoter to control GDH activity to about 3-times as high as that of the parent strain.

[0094]

Table 26

Strain	Glu (g/dl)	Asp (mg/dl)	Ala (mg/dl)	Relative activity of GDH	Relative value
AJ 13029	8.3	49	60	7.7	1.0
GA01	9.0	145	152	22.3	2.9
GA02	8.9	153	155	27.0	3.5
AJ13029/pGDH	8.6	201	190	82.7	10.7
AJ13029/pAJ220G	7.5	290	590	120.12	15.6

7. Drawings

FIG. 1 shows a flow of construction of GDH gene having a mutant promoter.

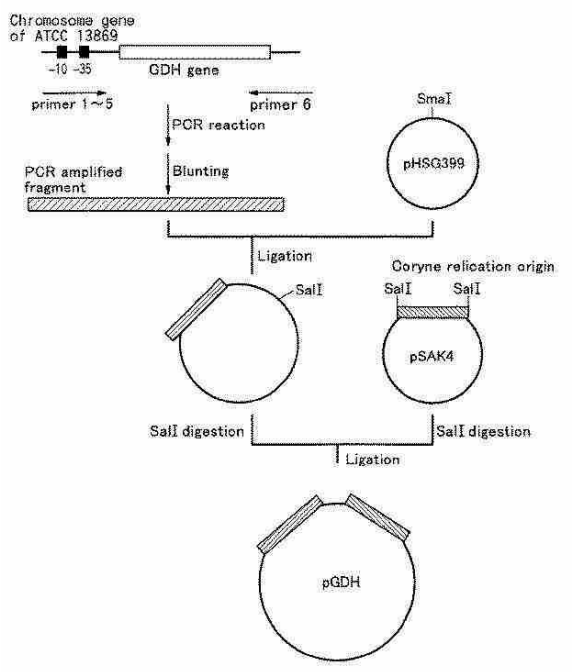


FIG. 2 shows a flow of construction of CS gene having a mutant promoter.

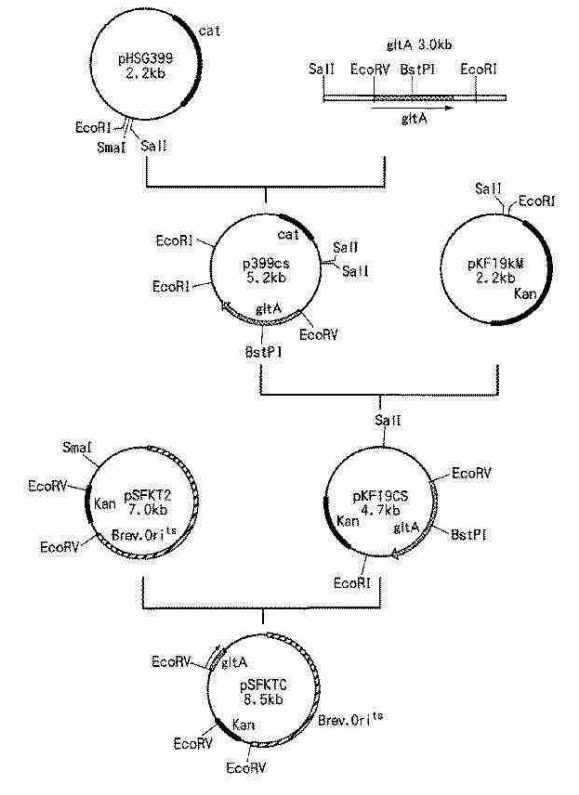
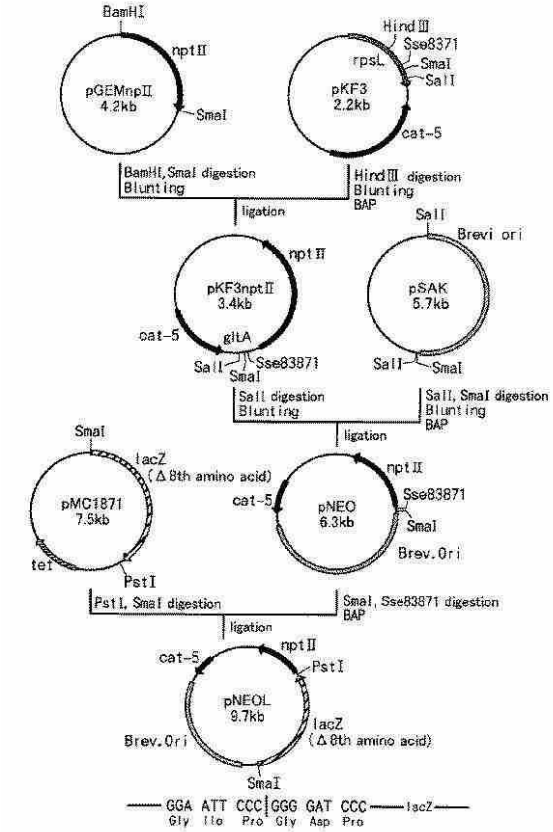


FIG. 3 shows a flow of construction of shuttle vector carrying lacZ as a reporter gene.



End

Attachment 13 "Statements in Description 2"

The detailed explanation of the invention in Description 2 contains the following statements:

1. Technical Field
[0001]

The present invention relates to the fermentation industry. In detail, the present invention relates to a process for producing L-glutamic acid and a bacterium used in this process. L-glutamic acid is widely used as a raw material in the production of seasonings and the like.

2. Background Art
[0002]

L-glutamic acid has been conventionally produced on an industrial scale by a fermentation method using coryneform bacteria which have L-glutamic acid-producing ability, such as bacteria belonging to the genus *Brevibacterium* or the genus *Corynebacterium*. As for these coryneform bacteria, strains isolated from nature or artificial mutants of such strains are used in order to enhance productivity.
[0003]

Generally, a wild-type strain of a coryneform bacterium does not produce L-glutamic acid under the condition where biotin is present. Accordingly, the production of L-glutamic acid by a coryneform bacterium is typically performed under a glutamic acid production-inducing condition such as a biotin-limited condition, a surfactant-added condition, and a penicillin-added condition (Non-Patent Document 1). Further, as strains which can produce L-glutamic acid in the presence of sufficient biotin without applying these methods, a surfactant-temperature-sensitive strain (Patent Document 1), a penicillin-sensitive strain (Patent Document 2), a cerulenin-sensitive strain (Patent Document 3), and a lysozyme-sensitive strain (Patent Document 4), etc. have been developed.
[0004]

However, these L-glutamic acid-producing bacteria which have been developed by these methods often showed a decrease in fatty acid-producing ability or a decrease in cell wall synthesis ability. Thus, it was highly probable that these strains caused reduced adaptation to environmental changes in exchange for L-glutamic acid production. Therefore, considerable efforts have been required for the development of a strain capable of accumulating a significant amount of L-glutamic acid when using these methods.

[0005]

On the other hand, a strain which produces L-glutamic acid in the presence of sufficient biotin can be achieved by deleting a gene encoding α-ketoglutarate dehydrogenase (Patent Document 5). However, the α-ketoglutarate dehydrogenase gene-deficient strain blocks the TCA cycle in the midway and thus grows slowly. Therefore, it was difficult to surely obtain a sufficient amount of bacterial cells, which was one of the problems.

[0006]

The yggB gene of a coryneform bacterium is a homologue of the yggB gene of *Escherichia coli* (Non-Patent Documents 2 and 3) and has been analyzed as a kind of mechanosensitive channel (Non-Patent Document 4). However, the effect of the yggB gene on L-glutamic acid production was not known.

3. Problem to be Solved by the Invention

[0007]

The problem to be solved by the present invention is to provide novel art to enhance the L-glutamic acid-producing ability of a coryneform bacterium when L-glutamic acid is produced by using the coryneform bacterium.

4. Means for Solving the Problem

[0008]

The present researchers have made extensive studies to solve the above problem. As a result, the present researchers have clarified that a yggB gene is involved in the production of L-glutamine by a coryneform bacterium. In addition, the present researchers have found that the L-glutamic acid-producing ability can be greatly enhanced by modifying the coryneform bacterium by using the yggB gene. Finally, the present researchers have completed the present invention.

5. Advantageous Effect of the Invention

[0010]

L-glutamic acid can be efficiently produced by using a coryneform bacterium which is modified by using a yggB gene of the present invention.

6. Mode for Carrying Out the Invention

(1) Coryneform Bacterium of the Present Invention

[0011]

... The coryneform bacterium of the present invention has an L-glutamic acid-producing ability, and is modified by using a yggB gene, and thereby the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain.

[0012]

In the present invention, the "coryneform bacterium" includes a bacterium which has been conventionally classified into the genus *Brevibacterium*, but is currently classified into the genus *Corynebacterium* (Int. J. Syst. Bacteriol., 41, 255(1991)), and also includes the *Brevibacterium* bacteria which are very closely related to *Corynebacterium* bacteria. Examples of such coryneform bacterium include the following.

... *Corynebacterium callunae*

Corynebacterium glutamicum ...

... *Corynebacterium melassecola* ...

... *Brevibacterium flavum* ...

[0013]

Specific examples of the coryneform bacteria are as follows:

... *Corynebacterium callunae* ATCC15991

Corynebacterium glutamicum ATCC13020, ATCC13032, ATCC13060 ...

... *Corynebacterium melassecola* ATCC17965 ...

... *Brevibacterium flavum* ATCC13826, ATCC14067 ...

[0015]

In the present invention, the phrase "L-glutamic acid-producing ability" means an ability to cause accumulation of L-glutamic acid in a medium or a bacterial cell when the coryneform bacterium of the present invention is cultured in the medium. The "L-glutamic acid-producing ability" may be a property of a wild-type strain of the coryneform bacterium, because most of the wild-type strains of coryneform bacterium can produce L-glutamic acid under the "L-glutamic acid-producing condition" as mentioned below. However, the L-glutamic acid-producing ability may be imparted or enhanced by breeding, or may be imparted by modification by using a yggB gene, as mentioned below.

The phrase "an L-glutamic acid-producing ability of a coryneform bacterium is enhanced" means that the L-glutamic acid-producing ability of the coryneform bacterium is enhanced as compared to a non-modified strain such as a wild-type strain. In this regard, examples of the wild-type strain of the coryneform bacterium include *Corynebacterium glutamicum* ATCC13032 strain, 13869 strain, 14067 strain, and *Corynebacterium melassecola* ATCC 17965 strain. The "non-modified strain" may also include a strain which expresses the wild-type yggB gene at the same level as the wild-type strains or a strain in which a mutation is not introduced into the coding region of a yggB gene.

[0029]

The coryneform bacterium of the present invention can be obtained by modifying the

above-described coryneform bacterium possessing L-glutamic acid-producing ability using a yggB gene so that the L-glutamic acid-producing ability is further enhanced. Alternatively, modification using a yggB gene may be performed first, followed by additional modification to impart or enhance L-glutamic acid-producing ability, as mentioned above.

Modification using a yggB gene includes enhancing the expression level of a yggB gene and introducing a mutant-type yggB gene, as described below.

(2) Enhancing Expression Level of yggB Gene

[0030]

... A yggB gene encodes a protein which is known as a kind of mechanosensitive channel, and which is also referred to as mscS (FEMS Microbiol Lett. 2003 Jan. 28; 218(2): 305-9.).

Enhancing the expression level of the yggB gene leads to improvement of the L-glutamic acid-producing ability of a coryneform bacterium as compared to a non-modified strain. That is, by culturing a coryneform bacterium which has been modified so that the expression level of the yggB gene is increased, the coryneform bacterium causes accumulation of a greater amount of L-glutamic acid than a non-modified strain, or alternatively, the coryneform bacterium produces L-glutamic acid at a higher rate than a non-modified strain. It is preferable that a yggB gene expression-enhancing strain increases the L-glutamic acid yield (a yield per consumed sugar) by 2% or more, more preferably 4% or more, and particularly preferably 6% or more, as compared to the parent strain or a non-modified strain. The yggB gene expression-enhancing strain may increase the bacterial cell production-subtracted yield as compared to a non-modified strain. The "bacterial cell production-subtracted yield" means a value calculated by subtracting the carbon yield which is used for production of bacterial cells from the yield per consumed sugar.

[0032]

The L-glutamic acid-producing ability of the coryneform bacterium which has been modified so that the expression level of the yggB gene increases may be enhanced as compared to a non-modified strain under at least one condition of an L-glutamic acid-producing condition and a condition with an excess of biotin.

In this regard, the "L-glutamic acid producing condition" means a condition in which a substance which induces L-glutamic acid production is added to a medium which contains carbon sources, nitrogen sources, inorganic salts, and, if necessary, organic micronutrients such as amino acids and vitamins, or alternatively a condition in which an amount of a substance which inhibits L-glutamic acid production is limited in a medium.

Examples of a substance which induces L-glutamic acid production include penicillin G and surfactants containing saturated fatty acid such as Tween 40. Examples of a substance which is limited due to inhibiting L-glutamic acid production include biotin ("Amino San Hakko (Amino Acid Fermentation)," Gakkai Shuppan Senta (Academic Society Publishing Center), 1986 (in Japanese)). Regarding the concentration of these substances which are added to the medium under the L-glutamic acid-producing condition, the concentration of biotin is less than 15 μg/L, preferably less than 10 μg/L, and more preferably less than 5 μg/L, and it is not necessary that any biotin is contained in the medium at all. The concentration of penicillin which is added to the medium is 0.1 U/ml or more, preferably 0.2 U/ml or more, and more preferably 0.4 U/ml or more. The concentration of surfactants which are added to the medium is 0.5 g/L or more, preferably 1 g/L or more, and more preferably 2 g/L or more.

On the other hand, the "condition with an excess of biotin" means, for example, a condition containing biotin of 30 μg/L or more, preferably 40 μg/L, and more preferably 50 μg/L, in the medium.

[0033] (Including a correction by Correction 2)

Examples of the yggB gene of the coryneform bacterium include a gene encoding a protein having an amino acid sequence of SEQ ID NO: 6, 62, 68, or 84. More specifically, examples of the yggB gene of the coryneform bacterium include: a gene having a sequence of nucleotide numbers 1437 to 3035 of SEQ ID NO: 5; a gene having a sequence of nucleotide numbers 507 to 2093 of SEQ ID NO: 61; a gene having a sequence of nucleotide numbers 403 to 2001 of SEQ ID NO: 67; and a gene having a sequence of nucleotide numbers 501 to 2099 of SEQ ID NO: 83. The gene having a sequence of nucleotide numbers 1437 to 3035 of SEQ ID NO: 5 is a yggB gene of *Corynebacterium glutamicum* ATCC13032 strain [Note by the Judgment: Corrected to "ATCC13869 strain" by Correction 2]. The gene having a sequence of nucleotide numbers 507 to 2093 of SEQ ID NO: 61 is a yggB gene of *Corynebacterium glutamicum* (*Brevibacterium flavum*) ATCC14967 strain. The gene having a sequence of nucleotide numbers 403 to 2001 of SEQ ID NO: 67 is a yggB gene of *Corynebacterium melassecola* ATCC17965 strain. The gene having a sequence of nucleotide numbers 501 to 2099 of SEQ ID NO: 83 is encoded to nucleotide numbers 1336092 to 1337693 in the genome sequence registered as GenBank Accession No. NC_003450 of *Corynebacterium glutamicum* ATCC13032, and is registered as NCgl 1221 (NP_600492. Reports small-conductance ... [gi:19552490]).

Further, since the nucleotide sequence of the yggB gene may differ depending on the species and the strain of the coryneform bacterium, the yggB gene may be a variant of

the nucleotide sequence consisting of nucleotide numbers 1437 to 3035 of SEQ ID NO: 5. A variant of the yggB gene can be searched by referring to the nucleotide sequence consisting of nucleotide numbers 1437 to 3035 of SEQ ID NO: 5; for example, by means of BLAST and the like (http://blast.genome.jp/). In addition, the variant of the yggB gene includes a gene which can be amplified by PCR using yggB gene homologues, for example, synthetic oligonucleotides of SEQ ID NOS: 75 and 76, for example, by using coryneform bacterium chromosome as templates. Further, as the gene of the present invention, a yggB gene of coryneform bacterium is preferable. However, a gene derived from other microorganisms may be used so far as the gene has a function in a coryneform bacterium. A mutant-type yggB gene mentioned below may be used as a gene of the present invention.

[0034]

The yggB gene may be a gene having an amino acid sequence in which one or several amino acids are substituted, deleted, inserted, or added in an amino acid sequence of SEQ ID NO: 6, 62, 68, or 84 so far as the yggB gene enhances the L-glutamic acid-producing ability of the coryneform bacterium. The number of "several" as referred to herein means, for example, 2 to 20, preferably 2 to 10, and more preferably 2 to 5.

The above substitution is preferably a conservative substitution. Examples of the conservative substitution include: substitution of ala with ser or thr; substitution of arg with gln, his, or lys; substitution of asn with glu, gln, lys, his, or asp; substitution of asp with asn, glu, or gln; substitution of cys with ser or ala; substitution of gln with asn, glu, lys, his, asp, or arg; substitution of glu with gly, asn, gln, lys, or asp; substitution of gly with pro; substitution of his with asn, lys, gln, arg, or tyr; substitution of ile with leu, met, val, or phe; substitution of leu with ile, met, val, or phe; substitution of lys with asn, glu, gln, his, or arg; substitution of met with ile, leu, val, or phe; substitution of phe with trp, tyr, met, ile, or leu; substitution of ser with thr or ala; substitution of thr with ser or ala; substitution of trp with phe or tyr; substitution of tyr with his, phe, or trp; and substitution of val with met, ile, or leu.

The yggB gene of the present invention includes a gene which encodes a protein which is at least 80% homology, preferably at least 90% homology, more preferably at least 95% homology, and particularly preferably at least 97% homology, to the entire amino acid sequence of SEQ ID NO: 6, 62, 68, or 84, and which enhances the L-glutamic acid-producing ability of the coryneform bacterium. In this regard, the homology can be calculated by BLAST developed by Karlin and Altschul (Pro. Natl. Acad. Sci. USA, 90, 5873 (1993)) and FASTA developed by Pearson (Methods Enzymol., 183, 63 (1990)). The homology search programs (such as BLASTN and BLASTP) based on these

algorithms are available from NCBI and others (//www.ncbi.nlm.nih.gov).

The substitutions, deletions, insertions, additions, or inversions of amino acids as mentioned above include those caused by naturally occurring mutants or variants, such as those caused by individual differences or by species differences in microorganisms carrying the yggB gene.

[0035]

Especially, the amino acids at the following positions in the amino acid sequence of SEQ ID NO: 6 may be substituted or deleted. The amino acid sequence of the YggB protein which is conserved among coryneform bacteria is shown in SEQ ID NO: 85, in which Xaa indicates a location where an amino acid may be substituted or deleted.

Glutamine residue at position 48 (preferably substituted with arginine residue)

Asparagine residue at position 275 (preferably substituted with serine residue)

Glutamic acid residue at position 298 (preferably substituted with alanine residue)

Alanine residue at position 343 (preferably substituted with valine residue)

Phenylalanine residue at position 396 (preferably substituted with isoleucine residue)

Serine residue at position 438 (preferably substituted with glycine residue)

Valine residue at position 445 (preferably substituted with alanine residue)

Alanine residue at position 454 (preferably substituted with valine residue)

Proline residue at position 457 (preferably substituted with serine residue)

Serine residue at position 474 (preferably substituted with asparagine residue)

Valine residue at position 517 (preferably deleted)

Glutamic acid residue at position 518 (preferably deleted)

Alanine residue at position 519 (preferably deleted)

Proline residue at position 520 (preferably deleted)

[0036]

The above yggB gene homologue can be obtained by modifying the gene having a sequence of nucleotide numbers 1437 to 3035 of the nucleotide sequence of SEQ ID NO: 5, the gene having a sequence of nucleotide numbers 507 to 2093 of the nucleotide sequence of SEQ ID NO: 61, the gene having a sequence of nucleotide numbers 403 to 2001 of the nucleotide sequence of SEQ ID NO: 67, or the gene having a sequence of nucleotide numbers 501 to 2099 of the nucleotide sequence of SEQ ID NO: 83 by, for example, the site-directed mutagenesis method, so that the amino acid residue at the specific site in the encoded protein is substituted, deleted, inserted, or added.

Further, such genes can also be obtained by a conventionally known mutagenesis treatment. Examples of the mutagenesis treatment include: a method of treating a gene having a sequence of nucleotide numbers 1437 to 3035 of the nucleotide sequence of SEQ

ID NO: 5, a gene having a sequence of nucleotide numbers 507 to 2093 of the nucleotide sequence of SEQ ID NO: 61, a gene having a sequence of nucleotide numbers 403 to 2001 of the nucleotide sequence of SEQ ID NO: 67, or a gene having a sequence of nucleotide numbers 501 to 2099 of the nucleotide sequence of SEQ ID NO: 83 in vitro with hydroxylamine, etc.; and treating a microorganism, for example, an *Escherichia* bacterium, carrying the gene with ultraviolet rays or a mutagenesis agent typically used in mutagenesis treatments, such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) or ethyl methanesulfonate (EMS). The above substitutions, deletions, insertions, additions, inversions, etc. of amino acids include those caused by naturally occurring mutants or variants, such as those caused by individual differences or by species differences in microorganisms carrying the yggB gene. When these genes are introduced into a coryneform bacterium, whether these genes enhance the L-glutamic acid-producing ability of coryneform bacterium can be confirmed by introducing the genes into a wild-type strain of the coryneform bacterium and determining if the L-glutamic acid-producing ability of the obtained coryneform bacterium is enhanced under the above condition, for example.

[0039]

Enhancing the expression of the above-described yggB gene can be attained by increasing the copy number of the yggB gene, modifying an expression regulatory sequence of the yggB gene, amplifying a transcriptional activator of the yggB gene, or reducing the activity of a transcriptional repressor of the yggB gene.

(3) Introduction of Mutant-Type yggB Gene

[0050]

... The modification using the yggB gene also includes introduction of a mutant-type yggB gene into a coryneform bacterium. "Introduction of a mutant-type yggB gene" as used herein includes: introduction of a mutation into a chromosomal yggB gene of a coryneform bacterium; introduction of a plasmid containing a mutant-type yggB gene into a coryneform bacterium; and substitution of a chromosomal yggB gene with a mutant-type yggB gene.

In the present invention, the "mutant-type yggB gene" means a yggB gene comprising a mutation in a coding region of the yggB gene, in which the mutation imparts a function to the yggB gene of enhancement of the L-glutamic acid-producing ability of a coryneform bacterium under a condition with an excess of biotin. In this regard, a mutant-type yggB gene may be a gene which enhances the L-glutamic acid-producing ability of a coryneform bacterium not only under a condition with an excess of biotin when the gene is introduced into the coryneform bacterium, but also even under the L-glutamic

acid-producing condition as mentioned above.

The phrase "enhance the L-glutamic acid-producing ability of a coryneform bacterium under a condition with an excess of biotin" means that when the coryneform bacterium of the present invention is cultured in a medium containing biotin at a concentration in which a non-modified strain of the coryneform bacterium cannot cause accumulation of L-glutamic acid, for example, in a medium containing 30 μg/L or more of biotin, the coryneform bacterium of the present invention causes accumulation of more L-glutamic acid in the medium than that of a non-modified strain, or the coryneform bacterium of the present invention produces L-glutamic acid at a higher rate than that of the non-modified strain.

[0051]

Hereinafter, a method for obtaining a mutant-type yggB gene of the present invention and a method for introducing a mutation into the yggB gene are stated. However, a method for obtaining a mutant-type yggB gene of the present invention and a method for introducing a mutation into the yggB gene are not limited to the following methods.

(II-1) Method of Utilizing odhA Gene-Deleted Strain

The inventors of the present invention have found that an odhA gene (a sucA gene)-deleted strain (hereinafter referred to as "odhA-disrupted strain") can be utilized for a method for obtaining a mutant-type yggB gene, in which the odhA gene encodes an E1o subunit of α-ketoglutarate dehydrogenase. The construction of the odhA-disrupted strain can be performed by a method using the sacB gene as mentioned above.

[0058]

(II-2) Method of Utilizing a Transposable Element

A coryneform bacterium having a mutant-type yggB gene may be also screened by using a transposable element which functions in the coryneform bacterium. The transposable element includes an insertion sequence (IS element) and a transposon. The mutant-type yggB gene may be a gene which can be obtained by allowing an insertion sequence (IS element) and/or a transposon to be accidentally inserted into the wild-type yggB gene. Alternatively, the mutant-type yggB gene may be a gene which is constructed artificially by using an artificial transposon. A strain into which a transposable element is inserted can be selected by reference to an index, such as a decrease in sensitivity to L-glutamic acid analogues. As an L-glutamic acid analogue, 4-fluoroglutamic acid can be utilized. Further, a strain into which a transposable element is inserted can be selected by selecting antibiotics-resistant strains at random with the use of an artificial transposon containing an antibiotics-resistant gene, and confirming the length of the yggB gene of the antibiotics-resistant strains by PCR.

[0064]

(II-3) Method of Introducing a Mutation into the yggB Gene In Vitro at Random

A mutation can be introduced into the yggB gene in vitro at random, among which the mutant-type yggB gene can be selected from a clone which can produce L-glutamic acid without the addition of surfactants. The parent strain useful for screening is preferably a strain which cannot cause accumulation of L-glutamic acid under the condition containing an excess of biotin; for example, *C. glutamicum* wildtype ATCC13869 strain, ATCC13032 strain, ATCC14067 strain, and *C. melassecola* wild-type ATCC17965 strain.

[0068]

(II-4) Method for Obtaining L-glutamic Acid Analogue-Resistant Strains

Mutant-type yggB genes can also be obtained by culturing a coryneform bacterium having a wild-type yggB gene in a medium containing an L-glutamic acid analogue, and obtaining L-glutamic acid analogue-resistant strains which can grow in the same medium. The parent strain used for screening is preferably the wild-type strain of the coryneform bacterium as mentioned above, and may be any strains having a wild-type yggB gene. In addition, a coryneform bacterium having a plasmid containing a wild-type yggB gene can also be used. ...

(4) Mutant-Type yggB Gene of the Present Invention

[0069]

Hereinafter, specific examples of the mutant-type yggB gene are stated. However, the mutant-type yggB gene of the present invention is not particularly limited so far as the mutant-type yggB gene has a mutation to enhance the L-glutamic acid producing ability of a coryneform bacterium under a condition in which an excess of biotin is present.

A. Mutation in C-Terminal Side

[0070]

... This mutation is a mutation which is introduced into a portion of a nucleotide sequence of a region encoding a sequence of amino acid numbers 419 to 533 of SEQ ID NO: 6, 68, 84, or 85, or a sequence of amino acid numbers 419 to 529 of SEQ ID NO: 62. For example, in the nucleotide sequence of SEQ ID NO: 5, this region corresponds to the region consisting of nucleotide numbers 2692 to 3035. This mutation may be of any type so far as the mutation is introduced into at least a portion of the nucleotide sequence of the region. Among mutations, insertion of an insertion sequence (hereinafter referred to as IS) or a transposon is preferable. The mutation may be accompanied by an amino acid substitution (missense mutation), or a frameshift mutation by insertion of the above IS may be introduced, or a nonsense mutation may be introduced, any of which is preferable.

(a) Mutation by Insertion of Transposable Element (2A-1-Type Mutation)

[0071]

... Examples of a mutation in the C-terminal side include a mutation in which an IS is inserted next to G at position 2691 of SEQ ID NO: 5. The nucleotide sequence of the mutant-type yggB gene in which this mutation is introduced is shown in SEQ ID NO: 7, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 8. In SEQ ID NO: 8, the region positioned downstream from valine at position 419 of SEQ ID NO: 6 is substituted with a sequence which is derived from a short IS. In this regard, the IS inserted into SEQ ID NO: 7 has high homology to IS1207 (GenBank accession No. X96962) and IS719 (GenBank accession No. E12759).

The above mutant-type yggB gene is called a 2A-1-type mutation. The 2A-1-type mutation includes mutation in which the above region of the C-terminal side in SEQ ID NOS: 6, 62, 68, 84, and 85 is deleted or substituted.

Further, the scope of the present invention includes a mutation in which another IS is inserted into the above region, for example, a mutation in which an IS encoding transposase as mentioned above is inserted into the above region. The position into which transposase is introduced may be any position in the above region, and a location which each transposase can easily recognize and a location of a hot spot into which an IS can be easily inserted are preferable.

(b) Mutation in which Proline Residue is Substituted with Another Amino Acid (66-Type Mutation, 22-Type Mutation)

[0072]

... In addition, examples of a mutation in the C-terminal side include a mutation in which proline which is present in a region in a sequence of amino acid numbers 419 to 533 of SEQ ID NO: 6, 68, 84, or 85, or a region of amino acid numbers 419 to 529 of SEQ ID NO: 62 is substituted with another amino acid. The proline which may be substituted is present in the following positions of SEQ ID NO: 6:

Proline residue at position 424 (424th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 437 (437th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 453 (453rd in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 457 (457th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 462 (462nd in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 469 (469th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 484 (484th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 489 (489th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 497 (497th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 515 (515th in SEQ ID NOS: 62, 68, 84, and 85);

Proline residue at position 529 (529th in SEQ ID NOS: 68, 84, and 85; and 525th in SEQ ID NO: 62); and

Proline residue at position 533 (533rd in SEQ ID NOS: 68, 84, and 85; and 529th in SEQ ID NO: 62).

It is considered that proline residues in the C-terminal side of the yggB gene play an important role in maintaining a three-dimensional structure of the YggB protein (Protein Eng. 2002 Jan; 15(1): 29-33, J Biol Chem. 1991 Dec 25; 266(36): 24287-94.).

Particularly, proline at position 424 or 437 is preferably substituted with another amino acid.

In this regard, another amino acid may be any amino acid so far as such other amino acid is a natural amino acid other than proline. Proline is preferably substituted into a residue selected from Lys, Glu, Thr, Val, Leu, Ile, Ser, Asp, Asn, Gln, Arg, Cys, Met, Phe, Trp, Tyr, Gly, Ala, and His. Particularly, proline at position 424 is preferably substituted with a hydrophobic amino acid Ala, Gly, Val, Leu, or Ile. Among these hydrophobic amino acids, proline at position 424 is more preferably substituted with a branched amino acid Leu, Val, or Ile (66-Type Mutation). Examples of a mutation in which proline at position 424 is substituted with leucine includes a mutation in which "C" at position 1673 is substituted with "T" in SEQ ID NO: 67. This mutant-type yggB gene is shown in SEQ ID NO: 69, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 70.

Further, proline at position 437 is preferably substituted with an amino acid having a hydroxyl group in its side chain (Thr, Ser, Tyr). Among these, proline at position 437 is most preferably substituted with Ser (22-Type Mutation). Examples of a mutation in which proline at position 437 is substituted with serine include a mutation in which C at position 2745 is substituted with T in SEQ ID NO: 5. In addition, this mutation may be accompanied with a mutation in which C at position 3060 is substituted with T in SEQ ID NO: 5. This mutant-type yggB gene is shown in SEQ ID NO: 73, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 74.

B. Mutation in Transmembrane Region

[0073]

... The YggB protein encoded by the yggB gene is presumed to have five transmembrane regions. In the amino acid sequence of the wild-type YggB protein shown in SEQ ID NOS: 6, 62, 68, 84, and 85, the transmembrane regions correspond to amino

acid numbers 1 to 23 (the first transmembrane region), amino acid numbers 25 to 47 (the second transmembrane region), amino acid numbers 62 to 84 (the third transmembrane region), amino acid numbers 86 to 108 (the fourth transmembrane region), and amino acid numbers 110 to 132 (the fifth transmembrane region). In SEQ ID NO: 5, DNA encoding these regions corresponds to nucleotide numbers 1437 to 1505, nucleotide numbers 1509 to 1577, nucleotide numbers 1620 to 1688, nucleotide numbers 1692 to 1760, and nucleotide numbers 1764 to 1832, respectively. The mutation of the present invention is preferably introduced into the DNA encoding these transmembrane regions. The mutation which is introduced into these regions is preferably a mutation including substitution, deletion, addition, insertion, or inversion of one or several amino acids without involving a frame-shift mutation and a nonsense mutation. Accordingly, in case of substitution in an amino acid sequence, a missense mutation involving amino acid substitution is preferable. The term "several" in the above-mentioned "substitution, deletion, addition, insertion, or inversion of one or several amino acids" means 2 to 20, preferably 2 to 10, more preferably 2 to 5, and still more preferably 2 or 3. The mutation of insertion and deletion of one or several amino acids includes introduction of point mutations of one to several nucleotides and introduction of nucleotide sequence without involving frame-shift mutation, and is, for example, insertion or deletion of 3, 6, 9, 12, 15, 18, or 21 nucleotides, preferably deletion or insertion of 3, 6, or 9 nucleotides, and more preferably deletion or insertion of 3 nucleotides.

(a) Mutation in the First Transmembrane Region (A1-Type Mutation)

[0074]

For example, the following mutations are listed.

... This mutation is a mutation in which one to several amino acids are inserted between leucine residue at position 14 and tryptophan residue at position 15 in an amino acid sequence shown in any of SEQ ID NOS: 6, 62, 68, 84, and 85.

Specific examples of the above mutation include a mutation in which three amino acids (for example, Cys-Ser-Leu) are inserted between leucine residue at position 14 and tryptophan residue at position 15. Examples of this mutation include a mutation in which TTCATTGTG is inserted next to G at position 1480 in SEQ ID NO: 5. The nucleotide sequence of the mutant-type yggB gene into which this mutation is introduced is shown in SEQ ID NO: 19, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 20.

(b) Mutation in the Fourth Transmembrane Region (19-Type Mutation)

[0075]

... This mutation is a mutation in which alanine residue at position 100 is substituted

with another amino acid in an amino acid sequence shown in any of SEQ ID NOS: 6, 62, 68, 84 and 85. "Another amino acid" may be any amino acid residue other than alanine, and means any of arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, methionine, leucine, lysine, phenylalanine, proline, serine, tryptophan, tyrosine, valine, and threonine. Among these, alanine residue at position 100 is preferably substituted with an amino acid having a hydroxyl group in its side chain (threonine, serine, tyrosine), and is particularly preferably substituted with threonine residue. Examples of such mutation include a mutation in which G at position 1734 is substituted with A in SEQ ID NO: 5.

The nucleotide sequence of the mutant-type yggB gene into which this mutation is introduced is shown in SEQ ID NO: 21, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 22.

(c) Mutation in the Fifth Transmembrane Region (L30-Type Mutation, 8-Type Mutation)

[0076]

... This mutation is a mutation in which alanine residue at position 111 is substituted with another amino acid in an amino acid sequence shown in any of SEQ ID NOS: 6, 62, 68, 84, and 85. "Another amino acid" may be any amino acid residue other than alanine, and means any of arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, methionine, leucine, lysine, phenylalanine, proline, serine, tryptophan, tyrosine, valine, and threonine. Among these, alanine residue at position 111 is preferably substituted with a branched amino acid (valine, isoleucine, leucine), particularly preferably valine residue, or with an amino acid having hydroxyl group in its side chain (threonine, serine, tyrosine), particularly preferably threonine residue. Examples of this mutation include a mutation in which C at position 1768 is substituted with T in SEQ ID NO: 5 (L30-type mutation) or a mutation in which G at position 837 is substituted with A in SEQ ID NO: 61 (8-type mutation). The nucleotide sequence of the mutant-type yggB gene into which L30-type mutation is introduced is shown in SEQ ID NO: 23, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 24. The nucleotide sequence of the mutant-type yggB gene into which 8-type mutation is introduced is shown in SEQ ID NO: 63, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 64.

C.

[0077]

The above-mentioned "mutant-type yggB gene," so far as it has a function capable of enhancing L-glutamic acid-producing ability under a condition in which an excess of

biotin is present, may be a functionally equivalent gene containing the mutation described above, for example, a gene that is substantially homologous to a mutant-type gene, such as DNA containing a sequence of nucleotide numbers 1437 to 2705 of SEQ ID NO: 7, DNA containing a sequence of nucleotide numbers 1437 to 3044 of SEQ ID NO: 19, DNA containing a sequence of nucleotide numbers 1437 to 3035 of SEQ ID NO: 21, DNA containing a sequence of nucleotide numbers 1437 to 3035 of SEQ ID NO: 23, DNA containing a sequence of nucleotide numbers 507 to 2093 of SEQ ID NO: 63, DNA containing a sequence of nucleotide numbers 403 to 2001 of SEQ ID NO: 69, or DNA containing a sequence of nucleotide numbers 548 to 2146 of SEQ ID NO: 73, that is capable of hybridizing to a polynucleotide containing a nucleotide sequence complementary to such sequence or to a probe having a part of that nucleotide sequence under stringent conditions. "Stringent conditions" as used herein are conditions under which a so-called specified hybrid is formed, and a non-specific hybrid is not formed. Examples of stringent conditions include typical Southern hybridization washing conditions, i.e. washing once or preferably two to three times at a salt concentration and temperature equivalent to 0.1×SSC, 0.1%SDS at 60°C, more preferably 0.1×SSC, 0.1%SDS at 68°C.

[0078]

As a protein which is encoded by the mutant-type yggB gene, so far as an activity of the protein has a function capable of enhancing L-glutamic acid-producing ability under a condition in which an excess of biotin is present, the protein may be a functionally equivalent protein which has, for example, an amino acid sequence in which one or several amino acids in addition to the amino acids of the above mutation point are substituted, deleted, inserted, or added in an amino acid sequence selected from SEQ ID NOS: 8, 20, 22, 24, 64, 70, and 74. The number of "several" as referred to herein means, for example, 2 to 20, preferably 2 to 10, more preferably 2 to 5. The above substitution is preferably a conservative substitution (neutral mutation). Examples of the conservative substitution include: substitution of ala with ser or thr; substitution of arg with gln, his, or lys; substitution of asn with glu, gln, lys, his, or asp; substitution of asp with asn, glu, or gln; substitution of cys with ser or ala; substitution of gln with asn, glu, lys, his, asp, or arg; substitution of glu with gly, asn, gln, lys, or asp; substitution of gly with pro; substitution of his with asn, lys, gln, arg, or tyr; substitution of ile with leu, met, val, or phe; substitution of leu with ile, met, val, or phe; substitution of lys with asn, glu, gln, his, or arg; substitution of met with ile, leu, val, or phe; substitution of phe with trp, tyr, met, ile, or leu; substitution of ser with thr or ala; substitution of thr with ser or ala; substitution of trp with phe or tyr; substitution of tyr with his, phe, or trp; and substitution of val with

met, ile, or leu. As mentioned above, the amino acid which is present in Xaa in SEQ ID NO: 85 may be substituted. The yggB gene of the present invention includes a homologue which encodes a protein which has at least 80% homology, preferably at least 90% homology, more preferably at least 95% homology, and particularly preferably at least 97% homology, to the entire amino acid sequence of SEQ ID NOS: 8, 20, 22, 24, 64, 70, and 74, and which encodes a protein having an activity which is equivalent to that of a protein encoded by the mutant-type yggB gene in the coryneform bacterium.

[0079]

Especially, the following amino acids may be substituted in the amino acid sequences of SEQ ID NO: 8, 80, 22, 24, 64, 70, or 74:

Glu at position 48 (preferably replaced by Arg)

Asp at position 275 (preferably replaced by Ser)

Glu at position 298 (preferably replaced by Ala)

Ala at position 343 (preferably replaced by Val)

Phe at position 396 (preferably replaced by Ile)

Ser at position 438 (preferably replaced by Gly)

Val at position 445 (preferably replaced by Ala)

Ala at position 454 (preferably replaced by Val)

Pro at position 457 (preferably replaced by Ser)

Ser at position 474 (preferably replaced by Asp)

Val at position 517 (preferably deleted)

Glu at position 518 (preferably deleted)

Ala at position 519 (preferably deleted)

Pro at position 520 (preferably deleted)

(5) Method of Introducing Mutant-Type yggB Gene into Coryneform Bacterium

[0080]

... The mutant-type yggB gene having the above mutation can be obtained by, for example, the site-directed mutagenesis method. More specifically, cloning can be performed by using an overlap extension PCR method, etc. which amplifies the relevant locations using a PCR primer having a sequence containing a mutation in the corresponding region of the yggB gene (Urban, A., Neukirchen, S. and Jaeger, K. E., A rapid and efficient method for site-directed mutagenesis using one-step overlap extension PCR. Nucleic Acids Res, 25, pp 2227-8. (1997).).

The coryneform bacterium of the present invention can be obtained by introducing the obtained mutant-type yggB gene into a coryneform bacterium. Examples of a method of introduction include a method of substituting a wild-type yggB gene on a chromosome

with the mutant-type yggB gene. The mutant-type yggB gene may be introduced into a strain in which a wild-type yggB gene on a chromosome is disrupted. In this regard, the mutant-type yggB gene may be introduced while a wild-type yggB gene is left on a chromosome as a single recombinant strain. One copy of the incorporated wild-type gene and one copy of the incorporated mutant-type gene may be present on a chromosome. The substitution of the yggB gene on the chromosome can be performed by using, for example, a temperature-sensitive plasmid containing a sacB gene encoding the above levan sucrase.

(6) Method of Producing L-glutamic Acid of the Present Invention

[0085]

... L-glutamic acid can be produced by obtaining a coryneform bacterium in the above manner, then culturing the obtained coryneform bacterium in a medium, to thereby cause production and accumulation of L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

[0086]

As the medium for the culture, an ordinary medium can be used where the ordinary medium contains carbon sources, nitrogen sources, and inorganic salts, and, if necessary, organic micronutrients such as amino acids and vitamins. Both synthetic medium and natural medium can be used. Any types of carbon sources and nitrogen sources may be used for the medium so far as such sources can be utilized by a strain to be cultured.

[0087]

As the carbon sources, saccharides such as glucose, glycerol, fructose, sucrose, maltose, mannose, galactose, starch hydrolysate, and molasses can be used. In addition, organic acids such as acetic acid and citric acid, and alcohols such as ethanol may also be used alone or in combination with other carbon sources. As the nitrogen sources, ammonia, ammonium salts such as ammonium sulfate, ammonium carbonate, ammonium chloride, ammonium phosphate, and ammonium acetate, or nitrates, and the like can be used. As the organic micronutrients, amino acids, vitamins, fatty acids, nucleic acids, peptone containing these substances, casamino acid, yeast extract, and soybean protein decomposition products can be used. When an auxotrophic mutant strain which requires an amino acid and the like for growth is used, such a required nutrient is preferably added. As the inorganic salts, phosphates, magnesium salts, calcium salts, iron salts, manganese salts, and the like can be used.

Further, surfactants and penicillin having appropriate concentrations may be added according to properties of the yggB gene-modified strain to be used. Furthermore, a concentration of biotin may be adjusted according to properties of the yggB gene-

modified strain to be used.

[0088]

As to the culture, aerobic culture is performed by preferably controlling the fermentation temperature to 20 to 45°C and pH to 3 to 9. When the pH decreases during the culture, neutralization should be performed by, for example, adding calcium carbonate or neutralizing with alkali such as ammonia gas. Under these conditions, culturing is performed for preferably about 10 to 120 hours, to thereby accumulate a considerable amount of L-glutamic acid in the culture solution.

[0089]

Further, the culture can also be performed while precipitating L-glutamic acid in the medium by using a liquid medium adjusted to conditions such that the produced L-glutamic acid precipitates. Examples of the conditions such that L-glutamic acid precipitates include a pH of 5.0 to 4.0, preferably a pH of 4.5 to 4.0, more preferably a pH of 4.3 to 4.0, particularly preferably a pH of 4.0 (Specification of European Patent Application Publication No. 1078989).

[0090]

Collection of L-glutamic acid from the culture solution after completion of the culture may be performed by conventional methods. For example, L-glutamic acid may be collected by removing bacterial cells from the culture solution, followed by concentrating and crystallizing L-glutamic acid, or by using ion exchange chromatography. When the culture is performed under conditions such that L-glutamic acid precipitates, the L-glutamic acid which is precipitated in the culture solution can be collected by centrifugation, filtration, etc. In this case, L-glutamic acid which is dissolved in the medium may also be isolated after crystallization of the dissolved L-glutamic acid.

7. Examples

(1) Working Example 1

[0092]

<Construction of Vector for Gene Disruption Carrying sacB Gene>

(A) Construction of pBS3

Construction of a vector for gene disruption carrying sacB gene was performed with reference to the method disclosed in International Publication Nos. WO 2005/113745 and WO 2005/113744. A sacB gene (SEQ ID NO: 11) was obtained by PCR using a chromosomal DNA of *Bacillus subtilis* as a template and SEQ ID NOS: 13 and 14 as primers. The PCR was performed using LA taq (TaKaRa) as follows: one cycle of heat retention at 94°C for 5 minutes; and 25 cycles of denaturing at 94°C for 30 seconds, annealing at 49°C for 30 seconds, and elongation at 72°C for 2 minutes. The obtained

PCR product was purified by a conventional method, and then digested with BglII and BamHI and blunt-ended. This fragment was digested with AvaII of pHSG299, and then was inserted into a blunt-ended site. The obtained DNA was used to transform competent cells of *Escherichia coli* JM109 (manufactured by Takara Bio Inc.). Then, the transformed bacterial cells were spread on LB medium containing 25 μg/ml kanamycin (hereinafter, abbreviated as Km) and were incubated overnight. Thereafter, colonies that appeared were selected, and single colonies were isolated to obtain transformants. Plasmids were extracted from the obtained transformants, and the plasmid having an insert of the objective PCR product was named pBS3. Figure 1 shows the procedure for constructing pBS3.

[0093]

(B) Construction of pBS4S

The SmaI site in the kanamycin-resistant gene sequence on pBS3 was modified by nucleotide substitution (a mutation which is not cut by SmaI) without causing amino acid substitution using cross-over PCR, to thereby obtain a plasmid in which kanamycin-resistant gene was disrupted. First, PCR was performed using pBS3 as a template and synthetic DNAs of SEQ ID NOS: 15 and 16 as primers, to thereby obtain an N-terminal amplified product of the kanamycin-resistant gene. On the other hand, in order to obtain a C-terminal side amplified product of the Km-resistant gene, PCR was performed using pBS3 as a template and synthetic DNAs of SEQ ID NOS: 17 and 18 as templates. PCR was performed using Pyrobest DNA Polymerase (manufactured by Takara Bio Inc.) as follows: one cycle of heat retention at 98°C for 5 minutes; and 25 cycles of denaturing at 98°C for 10 seconds, annealing at 57°C for 30 seconds, and elongation at 72°C for 1 minute, to obtain the objective PCR product. SEQ ID NOS: 16 and 17 are partially complementary to each other. Further, the SmaI site in these sequences was disrupted by nucleotide substitution without causing amino acid substitution. Then, in order to obtain a fragment of the mutant-type kanamycin-resistant gene in which the SmaI site was disrupted, the above N-terminal and C-terminal gene products were mixed together in substantially equimolar amounts. Thereafter, PCR was performed using the gene products as a template and synthetic DNAs of SEQ ID NOS: 15 and 18 as primers to obtain a Km-resistant gene amplified product in which a mutation was introduced. The PCR was performed using Pyrobest DNA Polymerase (manufactured by Takara Bio Inc.) as follows: one cycle of heat retention at 98°C for 5 minutes; and 25 cycles of denaturing at 98°C for 10 seconds, annealing at 57°C for 30 seconds, and elongation at 72°C for 1.5 minutes, to thereby obtain the objective PCR product.

[0094]

The PCR product was purified by a conventional method, and then digested with BanII, and thereafter inserted into the above BanII site of pBS3. This DNA was used to transform competent cells of *Escherichia coli* JM109 (manufactured by Takara Bio Inc.). Then, the transformed bacterial cells were spread on LB medium containing 25 μg/ml kanamycin and were incubated overnight. Thereafter, colonies that appeared were selected, and single colonies were isolated to obtain transformants. Plasmids were extracted from the obtained transformants, and the plasmid having an insert of the objective PCR product was named pBS4S. Figure 2 shows the procedure for constructing pBS4S.

(2) Working Example 2

[0095]

<Construction of odhA Mutant Strain Derived from *C. glutamicum* ATCC13869 Strain>

The sequence of the odhA gene encoding the α-ketoglutarate dehydrogenase of a coryneform bacterium has already been identified (Microbiology 142, 3347-3354, (1996), GenBank accession No. D84102). Based on the published sequence of the odhA gene, the primers stated in SEQ ID NOS: 1 and 2 were designed, and PCR was performed using the primers and the chromosomal DNA of ATCC13869 strain as a template to amplify only the internal sequence of the odhA gene. The amplified PCR fragment was completely digested with BamHI and inserted to the BamHI site of pBS4S constructed in Working Example 1, whereby the plasmid pBS4SΔsucAint was constructed (Figure 3 shows the constructing procedure).

[0096]

pBS4SΔsucAint was introduced into *C. glutamicum* ATCC13869 strain by the electric pulse method (Unexamined Patent Application Publication No. 1990-207791) and was spread over CM-Dex agar medium (5 g/l glucose, 10 g/l polypeptone, 10 g/l yeast extract, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4\text{-}5H_2O$, 3 g/l urea, 1.2 g/l soybean protein hydrolysate solution, and 20 g/l agar, adjusted to pH 7.5 with NaOH: autoclaved at 120°C for 20 minutes) containing 25 μg/ml kanamycin. After culturing at 31.5°C, PCR was performed using each of the chromosomes extracted from strains, which appeared to confirm that these strains were single cross-over recombinants in which pBS4SΔsucAint was incorporated by homologous recombination into the chromosome. Whether the strain is single cross-over recombinant can be easily confirmed by performing PCR using the chromosome of the candidate strain as a template, and a sequence (SEQ ID NO: 3) specific to pBS4S plasmid and a sequence (SEQ ID NO: 4) complementary to a chromosomal sequence as primers (the sequence of pBS4S is absent on chromosome of a non-recombinant strain, and thus,

no fragment is amplified from the non-recombinant strain, thereby enabling determination on whether the strain is single cross-over recombinant).

[0097]

The single cross-over recombinant thus obtained was named 2A-1 strain. The wild-type ATCC13869 strain and the 2A-1 strain were inoculated in 20 ml of a flask medium (30 g/l glucose, 15 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4\text{-}5H_2O$, 200 μg/l VB1, 300 μg/l biotin, and 0.48 g/l soybean hydrolysates (T-N), adjusted to pH 8.0 with KOH: autoclaved at 115°C for 10 minutes), followed by addition of 1 g of calcium carbonate which had been dry heat-sterilized beforehand, and each of the strains was cultured with shaking at 31.5°C. After the sugar was completely consumed, the concentration of L-glutamic acid in the medium was determined. The results are shown in Table 1 (OD620 is a turbidity diluted to 101 times, and indicates an amount of the bacterial cells; Glu (g/L) indicates an amount of accumulated L-glutamic acid). It was confirmed that the 2A-1 strain has an L-glutamic acid-producing ability under the condition where the parent ATCC13869 strain did not produce L-glutamic acid at all.

[0098]

[Table 1]

<Table 1 Amount of L-glutamic Acid Produced by 2A-1 Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.658	0.2
2A-1	0.315	17.7
Blank	0.002	0.4

(3) Working Example 3

[0099]

<Construction of odhA Revertant Strain Derived from 2A-1 Strain>

In the obtained 2A-1 strain, the odhA gene on the chromosome was disrupted by pBS4SΔT¥sucAint. By removing the plasmid on the chromosome from this strain, the odhA gene was reverted to the wild-type strain. On the other hand, the odhA-deleted strain grows very slowly in a medium containing no sugar. However, it is presumed that the odhA-revertant strain in which the odhA reverted to the wild-type grows well in a medium containing no sugar such as CM2B medium (10 g/l polypeptone, 10 g/l yeast extract, 5 g/l NaCl, 10 μg/L biotin, 20 g/l agar, adjusted to pH 7.0 with KOH). To obtain such a revertant strain, the 2A-1 strain was spread over a CM2B plate to select growth-improved strains. The growth-improved 2A-1R strain which thus appeared was purified

on the CM2B plate, and the kanamycin-sensitivity of the 2A-1R strain was examined. As a result, it was found that all of the selected strains were kanamycin-sensitive and sucrose-resistant.

[0100]

Since the pBS4SΔsucAint contains a kanamycin-resistant gene and the sacB gene encoding levan sucrase, strains harboring pBS4SΔsucAint exhibit kanamycin-resistance and sucrose-sensitivity, while strains from which pBS4SΔsucAint was removed exhibit kanamycin-sensitivity and sucrose-resistance. From these results, it was considered that the odhA had reverted to the wild-type one in the 2A-1R strain. Further, the sequence of the odhA gene was confirmed. As a result, it was confirmed that the odhA gene had no mutations. Therefore, it was concluded that the odhA had reverted to the wild-type [one] in the 2A-1R strain. The L-glutamic acid-producing ability of the 2A-1R strain was confirmed by a method similar to the method stated in Working Example 2. The results are shown in Table 2 (OD620 indicates an amount of the bacterial cells diluted to 101 times; Glu (g/L) indicates an amount of accumulated L-glutamic acid). Although the accumulation of L-glutamic acid by the 2A-1R strain was inferior to that by the 2A-1 strain, the 2A-1R strain accumulated a much higher amount of L-glutamic acid than the wild-type ATCC13869 strain (Table 2). In addition, when the shaking culture was continued after the sugar was completely consumed, decomposition of L-glutamic acid was observed in the 2A-1R strain, which has proved that the odhA gene had reverted to the wild-type in this strain (Figure 4).

[0101]

[Table 2]

<Table 2 Amount of L-glutamic Acid Produced by odhA Revertant Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.696	0.5
2A-1	0.332	17.1
2A-1R	0.327	14.3
Blank	0.002	0.6

(4) Working Example 4

[0102]

<Isolation of Gene which is Involved in L-glutamic Acid Production by 2A-1R Strain>

On the CM2B plate medium, 2A-1R strain could form colonies at substantially the same rate as that of the wild-type strain ATCC13869. However, on the minimum medium (20 g/l glucose, 2.64 g/L ammonium sulfate, 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 0.25 g/L

$MgSO_4 \cdot 7H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.01 g/L $MnSO_4 \cdot 7H_2O$, 0.01 g/L $CaCl_2$, 0.02 mg/L $CuSO_4$, 40 g/L MOPS, 30 mg/L protocatechuic acid, 200 μg/L VB1· HCl, 300 μg/L biotin, 20 g/L agar, adjusted to pH 6.7 with NaOH) plate, 2A-1R strain was found to show a considerably decreased colony-forming rate as compared to the wild-type strain ATCC13869. Accordingly, a gene which can recover the growth of the 2A-1R strain in the minimum medium was searched.

[0103]

The chromosomal DNA of ATCC13869 strain was partially digested with Sau3AI and ligated to the shuttle vector pVK9 which had been digested with BamHI. The obtained plasmid was precipitated with ethanol and used to transform electrocompetent cell of *E. coli* DH5α (Takara Bio Inc.) by the electric pulse method. pVK9 is a shuttle vector obtained by blunt-ending the AvaII site of pHSG299 (Takara Bio Inc.) and inserting therein a fragment comprising a sequence automatically replicable in coryneform bacteria excised with BamHI and KpnI from pHK4 (Unexamined Patent Application Publication No. 1993-007491). The transformed bacterial cells were spread over an LB plate medium (10 g/l polypeptone, 5 g/l yeast extract, 5 g/l NaCl, 20 g/l agar, adjusted to pH 7.0 with NaOH) containing 25 μg/ml kanamycin, and cultured at 37°C overnight. On the next day, all of the colonies which had appeared were collected from the plate with a platinum loop and plasmids were extracted to construct a library of ATCC13869. This library was transformed to the 2A-1R strain obtained in Working Example 3 by the electric pulse method, and the transformed cells were spread over a minimum medium plate containing 25 μg/ml kanamycin. The strains which showed an increased colony-forming rate were screened. By extracting a plasmid from the selected strains showing the increased colony-forming rate, it was found that the fragment having a sequence shown in SEQ ID NO: 5 was inserted into the BamHI site of pVK9. The obtained plasmid was named pL5k.

[0104]

Comparison of the nucleotide sequence inserted in the pL5k with the already published genome sequence of *Corynebacterium glutamicum* ATCC13032 (Acc. No. NC_003450) showed that pL5k contained only one ORF having the amino acid sequence shown in SEQ ID NO: 6 as a full-length ORF. Whether the ORF is a membrane protein can be predicted by the program "SOSUI" available on the internet (linked to http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0E.html as of October 7, 2004). Results of analysis of the ORF by using "SOSUI" suggested that five transmembrane regions are present. In the amino acid sequence of SEQ ID NO: 6, the transmembrane regions correspond to the regions of amino acid numbers 1 to 23, amino acid numbers 25 to 47,

amino acid numbers 62 to 84, amino acid numbers 86 to 108, and amino acid numbers 110 to 132. DNA sequences encoding these regions correspond to the nucleotide numbers 1437 to 1505, nucleotide numbers 1509 to 1577, nucleotide numbers 1620 to 1688, nucleotide numbers 1692 to 1760, and nucleotide numbers 1764 to 1832 of SEQ ID NO: 5. Each of the amino acid sequences of these regions is shown in SEQ ID NOS: 25 to 29 and Table 3.

[0105]

[Table 3]

<Table 3 Predicted Transmembrane Regions of YggB Protein>

No.	N terminal	transmembrane region	C terminal	type	length	Seq ID
1	1	MILGVPIQYLLYSLWNWIVDTGF	23	SECONDARY	23	25
2	25	VAIILVLAFLIPRIGRLAMRIIK	47	PRIMARY	23	26
3	62	QLAFAGVGVYIAQIVAFFMLAVS	84	PRIMARY	23	27
4	86	MQAFGFSLAGAAIPATIASAAIG	108	SECONDARY	23	28
5	110	GAQSIVADFLAGFFILTEKQFGV	132	SECONDARY	23	29

(5) Working Example 5

[0107]

<Identification of Mutation Point of yggB Gene of 2A-1R Strain>

pL5K complemented the growth of the 2A-1R strain in the minimum medium, which suggested the possibility that the yggB gene of the 2A-1R strain has some mutations. Accordingly, a nucleotide sequence of the yggB gene of the 2A-1R strain was determined. As a result, it was found that in the 2A-1R strain, an IS was inserted into the C-terminal side region of the yggB gene (Figure 5). The nucleotide sequence of the mutant-type yggB gene of the 2A-1R strain is shown in SEQ ID NO: 7 and the corresponding amino acid sequence is shown in SEQ ID NO: 8. This suggested the possibility that the L-glutamic acid-producing ability of the 2A-1R strain was maintained by the mutation in the yggB gene. In this regard, this mutation was present not only in the 2A-1R strain, but also in the 2A-1 strain. This mutation is presumed to have occurred as a suppressor mutation to stably excrete L-glutamic acid out of the cell when the mutation was introduced into the odhA gene. The mutation in which an IS was inserted was named 2A-1-type mutation.

(6) Working Example 6

[0108]

<Construction of 2A-1-Type yggB Mutant Strain and Evaluation of L-glutamic Acid-Producing Ability>

(6-1) Introduction of 2A-1-Type Mutation into Wild-Type Strain, and Evaluation Thereof (Single Cross-Over Recombinant Strain)

PCR was performed using the chromosomal DNA of the 2A-1 strain as a template and synthetic DNAs shown in SEQ ID NOS: 9 and 10 as primers to amplify a fragment of yggB gene having the 2A-1-type mutation. The amplified product was digested with SacI and inserted into the SacI site of pBS3 of Working Example 1. A plasmid in which a yggB fragment having 2A-1-type mutation was cloned was named pBS3yggB2A.

The pBS3yggB2A was introduced into *C. glutamicum* ATCC13869 strain by the electric pulse method and was spread over a CM-Dex agar medium containing 25 μg/ml kanamycin. The strains that appeared after culturing at 31.5°C were evaluated by PCR to confirm that they are single cross-over recombinants in which pBS3yggB2A was incorporated into the chromosome by homologous recombination.

The obtained single cross-over recombinant strain was named 13869-2A strain. In this strain, the wild-type yggB gene and the mutant-type yggB gene were both expressed.

[0109]

The L-glutamic acid-producing ability of the obtained yggB mutation-introduced strain 13869-2A was confirmed by the method stated in Working Example 2. The results are shown in Table 4 (OD620 indicates an amount of the bacterial cells diluted to 101 times; Glu (g/L) indicates an amount of accumulated L-glutamic acid). It could be confirmed that 13869-2A strain clearly had an L-glutamic acid-producing ability even under the condition in which ATCC13869 strain did not produce L-glutamic acid. From these results, it has been shown that the mutation in the yggB gene can induce L-glutamic acid production.

[0110]

[Table 4]

<Table 4 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.625	0.3
2A-1R	0.334	15.5
13869-2A	0.582	3.6
Blank	0.002	0.6

[0111]

(6-2) Introduction of 2A-1-Type Mutation into Wild-Type Strain, and Evaluation Thereof (Double Cross-Over Recombinant Strain)

In order to construct a strain having only the mutant-type gene, 13869-2A strain was cultured on a CM-Dex liquid medium overnight and the obtained suspension was spread over an S10 agar medium (100 g/l sucrose, 10 g/l polypeptone, 10 g/l yeast extract, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4\text{-}5H_2O$, 3 g/l urea, 1.2 g/l soybean protein hydrolysate solution, 20 g/l agar, adjusted to pH 7.5 with NaOH: autoclaved at 120°C for 20 minutes) and cultured at 31.5°C. Among the colonies that appeared, the strain exhibiting sensitivity to kanamycin was purified on an s2B agar medium. Chromosomal DNAs were prepared from these strains. Then, PCR was performed using synthetic DNAs shown in SEQ ID NOS: 9 and 10 as primers to confirm the mutation. The strain in which an IS-like sequence was inserted into the yggB gene was named 13869-2A-7 strain.

[0112]

The L-glutamic acid-producing ability of the obtained 13869-2A-7 strain was confirmed by the method stated in Working Example 2. The results are shown in Table 5 (OD620 indicates an amount of the bacterial cells diluted to 101 times; Glu (g/L) indicates an amount of accumulated L-glutamic acid). It was shown that the 13869-2A-7 strain has the L-glutamic acid-producing ability which was equivalent to or higher than that of the 2A-1R strain, from which it could be confirmed that L-glutamic acid production in the presence of an excess of biotin was caused by the mutation in the yggB gene.

[0113]

[Table 5]

<Table 5 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene Chromosome-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.648	0.4
2A-1R	0.420	13.8
13869-2A-7	0.414	16.1
Blank	0.002	0.7

(7) Working Example 7

[0114]

<Construction of A1-Type YggB Mutant Strain, and Evaluation Thereof>

The above L-glutamic acid-producing odhA mutant strains were analyzed. As a result,

five types of mutations were identified on the yggB gene in addition to the above 2A-1 mutation. Hereinafter, these mutations are called A1-type mutation, 19-type mutation, L30-type mutation, 8-type mutation, and 66-type mutation, respectively. Each of the former three mutations, i.e., the A1-type mutation, 19-type mutation, and L30-type mutation, was introduced into the chromosome of ATCC13869 strain, and effects of these mutations were confirmed. In addition, the 8-type mutation was introduced into the chromosome of the ATCC14067 strain, and the effect of the mutation was confirmed. Further, the 66-type mutation was introduced into the chromosome of the *C. melassecola* ATCC17965 strain, and the effect of the mutation was confirmed.

[0115]

The A1-type mutation is a mutation in which TTCATTGTG is inserted next to G at position 1480 in SEQ ID NO: 5, and a mutation in which cysteine-serine-leucine residues are inserted between leucine residue at position 14 and tryptophan residue at position 15 in SEQ ID NO: 6. The nucleotide sequence of the mutant-type yggB gene into which this mutation is introduced is shown in SEQ ID NO: 19, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 20.

[0116]

The A1-type mutant gene can be obtained as follows. PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 30 and 31 as primers and the chromosomal DNA of ATCC13869 strain as a template to prepare an N-terminal side fragment. Similarly, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 32 and 33 as primers to prepare a C-terminal side fragment. Subsequently, PCR can be performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 9 and 34 as primers to obtain a partial fragment of the A1-type mutant yggB gene. The obtained YggB fragment can be digested with SacI and inserted into the SacI site of pBS4S to construct a plasmid for introducing this type of mutation. pBS4 YggBA1 thus obtained is introduced into the chromosome of ATCC13869 strain by a method similar to the method stated in Working Example 6, and then removed. The sequence of the yggB gene of the obtained kanamycin-sensitive strain can be determined and the strain having the A1-type substituted yggB gene is selected, to thereby construct a strain having A1-type mutation. The A1-type mutant strain having these mutations was named ATCC13869-A1 strain.

[0117]

ATCC13869-A1 strain and the parent ATCC13869 strain were cultured by a method similar to the method stated in Working Example 2. After completion of the culture, the

amount of L-glutamic acid contained in the culture solution was measured by a known method.

The ATCC13869-A1 strain in which the A1-type mutation was introduced on the chromosome greatly enhanced L-glutamic acid accumulation as compared to the parent ATCC13869 strain.

[0118]

[Table 6]

<Table 6 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene (A1) Chromosome-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.650	0.5
ATCC13869-A1	0.548	8.6

(8) Working Example 8

[0119]

<Construction of 19-Type YggB Mutant Strain, and Evaluation Thereof>

The 19-type mutation is a mutation in which G at position 1734 is substituted with A in SEQ ID NO: 5, and a mutation in which alanine at position 100 is substituted with threonine in the amino acid sequence of SEQ ID NO: 6. The nucleotide sequence of the mutant-type yggB gene into which this mutation is introduced is shown in SEQ ID NO: 21, and the amino acid sequence of the mutant-type YggB protein encoded by this gene is shown in SEQ ID NO: 22. By a method similar to the method stated in Working Example 7, the 19-type mutation-introduced yggB mutant strain is constructed. Specifically, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 30 and 35 as primers and the chromosomal DNA of ATCC13869 strain as a template to prepare an N-terminal side fragment. Similarly, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 33 and 36 as primers to prepare a C-terminal side fragment. Subsequently, PCR can be performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 9 and 34 as primers to obtain a partial fragment of the 19-type yggB gene. The obtained yggB fragment can be digested with SacI and inserted into the SacI site of pBS4S to construct a plasmid for introducing this type of mutation. pBS4 YggB19 thus obtained is introduced into the chromosome of ATCC13869 strain by a method similar to the method stated in Working Example 6, and then removed. The sequence of the yggB gene of the obtained kanamycin-sensitive strain can be determined and the strain having the 19-type substituted yggB gene is selected, to thereby construct

a strain having 19-type mutation. The 19-type mutant strain having these mutations was named ATCC13869-19 strain.

[0120]

ATCC13869-19 strain and the parent ATCC13869 strain were cultured by a method similar to the method stated in Working Example 2. After completion of the culture, the amount of L-glutamic acid contained in the culture solution was measured by a known method. The ATCC13869-19 strain in which the 19-type mutation was introduced on the chromosome greatly enhanced L-glutamic acid accumulation as compared to the parent ATCC13869 strain.

[0121]

[Table 7]

<Table 7 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene (19 Mutation) Chromosome-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.650	0.5
ATCC13869-19	0.614	0.7

(9) Working Example 9

[0122]

<Construction of L30-Type YggB Mutant Strain >

The L30-type mutation is a mutation in which C at position 1768 is substituted with T in SEQ ID NO: 5, and a mutation in which alanine at position 111 is substituted with valine in SEQ ID NO: 6. The nucleotide sequence of the mutant-type yggB gene in which this mutation is introduced is shown in SEQ ID NO: 23, and the amino acid sequence of the mutant-type YggB protein encoded by this gene is shown in SEQ ID NO: 24.

By a method similar to the method stated in Working Example 7, the L30-type mutation-introduced yggB mutant strain is constructed. Specifically, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 30 and 37 as primers and the chromosomal DNA of ATCC13869 strain as a template to prepare an N-terminal side fragment. Similarly, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 34 and 38 as primers to prepare a C-terminal side fragment. Subsequently, PCR was performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 9 and 34 as primers to obtain a partial fragment of the L30-type yggB gene. The obtained mutant-type yggB fragment is digested with SacI and inserted into the SacI site of pBS4S to construct a plasmid for introducing this type of mutation. pBS4 YggB-L thus

obtained was introduced into the chromosome of ATCC13869 strain by a method similar to the method stated in Working Example 6, and then removed. The sequence of the yggB gene of the obtained kanamycin-sensitive strain can be determined and the strain having the L30-type substituted yggB gene is selected, to thereby construct a strain having L30-type mutation. The L30-type mutant strain having these mutations was named ATCC13869-L strain.

[0123]

ATCC13869 strain and ATCC13869-L strain were cultured by a method similar to the method stated in Working Example 2. After completion of the culture, the amount of L-glutamic acid contained in the culture solution was measured by a known method. The results are shown in Table 8 (OD620 indicates an amount of the bacterial cells diluted to 101 times; Glu (g/L) indicates an amount of accumulated L-glutamic acid). ATCC13869-L30 strain into which L-30 mutation was introduced greatly enhanced L-glutamic acid accumulation as compared to the parent ATCC13869 strain.

[0124]

[Table 8]

<Table 8 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene (L-30 Mutation) Chromosome-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.650	0.5
ATCC13869-L30	0.389	15.9

(10) Working Example 10

[0125]

<Culture of the Mutant-Type yggB Gene-Introduced Strains Under L-glutamic Acid-Producing Conditions, and Evaluation Thereof>

L-glutamic acid production of a coryneform bacterium can be induced by adding fatty acid-based surfactants such as Tween 40 or by limiting biotin. Accordingly, the ATCC13869 strain and the ATCC13869-19 strain were also cultured under Tween40-added condition and biotin-limited condition, respectively. For seed culture, each of the strains was inoculated into 20 ml of flask medium (80 g/l glucose, 30 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4$-$5H_2O$, 200 μg/l VB1, 60 μg/l biotin, 0.48 g/l soybean hydrolysates (T-N), adjusted to pH 8.0 with KOH: autoclaved at 115°C for 10 minutes), and then 1 g of calcium carbonate which had been dry heat-sterilized beforehand was added thereto, followed by a shaking culture at 31.5°C. The culture solution obtained after complete consumption of sugars

was used as a seed culture solution. For Tween40-added culture, 2 ml of the seed culture solution was inoculated into 20 ml of the flask medium (80 g/l glucose, 30 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4$-$5H_2O$, 200 μg/l VB1, 60 μg/l biotin, 0.48 g/l soybean hydrolysates (T-N), adjusted to pH 8.0 with KOH: autoclaved at 115°C for 10 minutes), and then 1 g of calcium carbonate which had been dry heat-sterilized beforehand was added thereto, followed by a shaking culture at 31.5°C. At the time when OD620 (× 101) reached 0.2 after the start of culture, Tween 40 was added in order to attain a final concentration of 5 g/L, and then the culture was continued. For biotin-limited culture, 1 ml of the seed culture solution was inoculated into 20 ml of the flask medium (80 g/l glucose, 30 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4$-$5H_2O$, 200 μg/l VB1, 0.48 g/l soybean hydrolysates (T-N), adjusted to pH 8.0 with KOH: autoclaved at 115°C for 10 minutes), and then 1 g of calcium carbonate which had been dry heat-sterilized beforehand was added thereto, followed by a shaking culture at 31.5°C. Under these culture conditions, the final concentration of biotin became about 2.9 μg/L.

After 40 hours from the start of culture, the concentrations of L-glutamic acid in the medium were measured for both of the Tween40-added culture and the biotin-limited culture. The results are shown in Table 9 (OD620 indicates an amount of the bacterial cells diluted to 101 times; Glu (g/L) indicates an amount of accumulated L-glutamic acid). It has been found that ATCC13869-19 strain increased L-glutamic acid accumulation even under L-glutamic acid-producing condition.

[0126]

[Table 9]

<Table 9 Amount of L-glutamic Acid Produced by Mutant-Type yggB Gene-Introduced Strain under the Glutamic Acid Production-Inducing Condition>

	OD620 (x101)	Glu (g/L)
Tween40-added		
ATCC13869	0.538	25.6
ATCC13869-19	0.395	28.6
biotin-limited		
ATCC13869	0.462	36.0
ATCC13869-19	0.431	40.0

[0127]

The ATCC13869 strain, the ATCC13869-L30 strain, the ATCC13869-A1 strain, the ATCC13869-19 strain, and the strain in which yggB was amplified by plasmid were also

cultured with Tween 40 added. Each of these strains were cultured on a CM-Dex plate medium overnight, and cells collected from 1/6 area of the plate were inoculated in 20 ml of a flask medium (80 g/l glucose, 30 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4\text{-}5H_2O$, 200 μg/l VB1, 60 μg/l biotin, and 0.48 g/l soybean hydrolysates (T-N), adjusted to pH 8.0 with KOH: autoclaved at 115°C for 10 minutes), followed by addition of 1 g of calcium carbonate which had been dry heat-sterilized beforehand, and each of the strains was cultured with shaking at 31.5°C. After 5 hours from the start of culture, Tween 40 was added in order to attain a final concentration of 1 g/L. After 24 hours from the start of culture, the amount of bacterial cells and the yield of L-glutamic acid were each analyzed. As a result, as shown in Table 10, it is shown that all of the ATCC13869-19 strain, the ATCC13869-L30 strain, the ATCC13869-A1 strain, and the wild-type yggB gene-amplified strain (ATCC13869/pL5k-1) enhanced the yield of L-glutamic acid under L-glutamic acid-producing condition.

[0128]

[Table 10]

<Table 10 Amount of L-glutamic Acid Produced by Each Strain in Tween40-Added Culture>

	OD620 (x101)	Glu (g/L)
ATCC13869	0.887	12.8
ATCC13869/pVK9	0.748	12.4
ATCC13869/pL5k-1	0.711	19.2
ATCC13869-19	0.786	21.0
ATCC13869-A1	0.629	34.9
ATCC13869-L30	0.649	28.3
Blank	0.001	0.5

(11) Working Example 11

[0129]

<Construction of 8-Type YggB Mutant Strain>

The 8-type mutation is a mutation in which G at position 837 is substituted with A in SEQ ID NO: 61, and a mutation in which alanine at position 111 is substituted with threonine in SEQ ID NO: 62. The nucleotide sequence of the mutant-type yggB gene into which this mutation is introduced is shown in SEQ ID NO: 63, and the amino acid sequence of the mutant-type YggB protein encoded by this gene is shown in SEQ ID NO: 64.

By a method similar to the method stated in Working Example 7, the 8-type mutation-introduced yggB mutant strain is constructed. Specifically, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 30 and 65 as primers and the chromosomal DNA of *Brevibacterium flavum*ATCC14067 strain as a template to prepare an N-terminal side fragment. Similarly, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 34 and 66 as primers to prepare a C-terminal side fragment. Subsequently, PCR is performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 9 and 34 as primers to obtain a partial fragment of the 8-type yggB gene. The obtained mutant-type yggB fragment was digested with SacI and inserted into the SacI site of pBS4S to construct a plasmid for introducing this type of mutation. pBS4 YggB8 thus obtained is introduced into the chromosome of ATCC14067 strain by a method similar to the method stated in Working Example 6, and then removed. The sequence of the yggB gene of the obtained kanamycin-sensitive strain is determined and the strain having the 8-type substituted yggB gene is selected, to thereby construct a strain having 8-type mutation. The 8-type mutant strain having these mutations is named ATCC14067yggB8 strain.

(12) Working Example 12

[0130]

<Construction of 66-Type YggB Mutant Strain>

The 66-type mutation is a mutation in which C at position 1673 is substituted with T in SEQ ID NO: 67, and a mutation in which proline at position 424 is substituted with leucine in SEQ ID NO: 68. The nucleotide sequence of the mutant-type yggB gene in which this mutation is introduced is shown in SEQ ID NO: 69, and the amino acid sequence of the mutant-type YggB protein encoded by this gene is shown in SEQ ID NO: 70.

By a method similar to the method stated in Working Example 7, the 66-type mutation-introduced yggB mutant strain is constructed. Specifically, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 30 and 71 as primers and the chromosomal DNA of *C. melassecola* ATCC17965 strain as a template to prepare an N-terminal side fragment. Similarly, PCR is performed by using the synthetic DNAs shown in SEQ ID NOS: 34 and 72 as primers to prepare a C-terminal side fragment. Subsequently, PCR can be performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 9 and 10 as primers to obtain a partial fragment of the 66-type yggB gene. The obtained mutant-type yggB fragment was digested with SacI and

inserted into the SacI site of pBS4S to construct a plasmid for introducing this type of mutation. pBS4 YggB66 thus obtained is introduced into the chromosome of ATCC17965 strain by a method similar to the method stated in Working Example 6, and then removed. The sequence of the yggB gene of the obtained kanamycin-sensitive strain can be determined and the strain having the 66-type substituted yggB gene is selected, to thereby construct a strain having 66-type mutation. The 66-type mutant strain having these mutations is named yggB66 strain.

(13) Working Example 13

[0131]

<Screening of the Mutant-Type yggB Genes by In Vitro Mutation>

(13-1) Construction of yggB-Deleted Strain

Mutant-type yggB genes can also be obtained by introducing a mutation into yggB in vitro at random, and selecting a clone capable of producing L-glutamic acid without the addition of surfactants. In order to perform screening for mutant-type genes, a yggB gene-deleted strain was constructed first. PCR was performed by using the synthetic DNAs shown in SEQ ID NOS: 39 and 40 as primers and the chromosomal DNA of ATCC13869 strain as a template to prepare an N-terminal side fragment. Similarly, PCR was performed by using the synthetic DNAs shown in SEQ ID NOS: 41 and 42 as primers to prepare a C-terminal side fragment. SEQ ID NO: 40 and SEQ ID NO: 41 are complementary to each other. Subsequently, PCR was performed by using a mixture of an equal amount of the N-terminal side fragment and the C-terminal side fragment as a template and the synthetic DNAs shown in SEQ ID NOS: 39 and 42 as primers to obtain a fragment in which an ORF of the yggB gene was deleted.

[0132]

The obtained PCR fragment was digested with SacI and inserted into the SacI site of pBS4S to construct a plasmid for introducing deletion mutation. pBS4ΔYggB thus obtained is introduced into the chromosome of ATCC13869 strain by the method stated in Working Example 6, and then removed. PCR was performed by using the chromosomal DNA of the obtained kanamycin-sensitive strain as a template and the synthetic DNAs of SEQ ID NOS: 39 and 42 as primers to confirm that the yggB gene was deleted. The obtained yggB-deleted strain was named ATCC13869ΔyggB strain.

[0133]

(13-2) In Vitro Screening of Mutant-Type yggB Genes

On the other hand, mutagenesis treatment on the yggB gene was performed as follows. First, since the plasmid pL5k contains some regions other than the yggB gene, pL5k was treated with XhoI and SalI, and then self-ligated to obtain the plasmid pL5kXS. A SalI

recognition site is not present in the nucleotide sequence of SEQ ID NO: 5, but is present in the multi-cloning site of pBS3. About 10 μg of the obtained pL5kXS was dissolved in 500 mM phosphate buffer, 400 mM hydroxylamine, and 1 mM EDTA (pH 6.0), and then heated at 75°C for 30 to 90 minutes to introduce a mutation. The plasmid after mutation introduction was desalted using SUPREC-02 (manufactured by Takara Bio Inc.), and then introduced into ATCC13869ΔyggB strain by the method stated in Working Example 6. Thereafter, transformed cells were selected in the CM2B medium containing 25 μg/ml Km. On the other hand, pL5kXS before mutagenesis treatment was introduced into the ATCC13869ΔyggB strain as a control. The appeared transformants are inoculated into 2 ml of a liquid CM2BGU2 medium (CM2B medium stated in Working Example 3 further containing 10 g/L glucose and 15 g/L urea) and cultured at 31.5°C for 5 hours with shaking, followed by determining a concentration of glutamic acid.

The strain obtained by transforming the ATCC13869ΔyggB strain with the mutated pL5kXS was cultured in the CM2BGU2 medium in vitro. The evaluation results of the culture are shown in Table 11. There were three clones which cause accumulation of 1 g/L or more of L-glutamic acid among the transformants obtained from a plasmid mixture with a mutagenesis treatment time of 60 minutes and 90 minutes. The amount of L-glutamic acid contained in the starting medium was 0.16 g/L, and the amount of L-glutamic acid which had accumulated by the ATCC13869ΔyggB/pL5kXS (without mutagenesis treatment) strain was 0.31 g/L.

The strain obtained by transforming the ATCC13869ΔyggB strain with pL5kXS was cultured in the CM2BGU medium (the same composition as CM2BGU2 medium, except that urea concentration is 1.5 g/L) in vitro. The evaluation results of the culture are shown in Table 12. One clone which causes accumulation of 1 g/L or more of L-glutamic acid was obtained among the transformants obtained from a plasmid mixture with a mutagenesis treatment time of 90 minutes.

[0134]

[Table 11]

<Table 11 Amount of L-glutamic Acid Produced by yggB Plasmid Subjected to Mutagenesis Treatment - 1>

Amount of Accumulated Glu (g/L)	Number of Clones		
	Mutagenesis Treatment Time		
	30 min	60 min	90 min
Glu≤0.4	40	36	39
0.4<Glu≤0.6	8	11	6
0.6<Glu≤0.8	0	0	1

0.8<Glu≤1	0	0	0
1<Glu	0	1	2

[0135]

[Table 12]

<Table 12 Amount of L-glutamic Acid Produced by yggB Plasmid Subjected to Mutagenesis Treatment - 2>

Amount of Accumulated Glu (g/L)	Number of Clones	
	Mutagenesis Treatment Time	
	60 min	90 min
Glu≤0.7	45	41
0.7<Glu≤0.9	2	7
0.9<Glu	1	0

[0136]

A plasmid which gained a producing ability to accumulate 1 g/L or more of L-glutamic acid by mutagenesis treatment for 60 minutes as shown in Table 11 was named pL5kXSm-22. A plasmid which gained a producing ability to accumulate 0.9 g/L or more of L-glutamic acid by mutagenesis treatment for 90 minutes as shown in Table 12 was named pL5kXSm-27. ATCC13869ΔyggB/pL5kXS strain, ATCC13869ΔyggB/ pL5kXSm-27 strain, and ATCC13869ΔyggB/pL5kXSm-22 strain were cultured under the conditions stated in Working Example 2, and the amounts of bacterial cells and L-glutamic acid accumulation after 4 hours were analyzed. Table 13 shows the mean value of three independent experiments. It could be confirmed that the ATCC13869ΔyggB/pL5kXSm-27 strain and the ATCC13869ΔyggB/pL5kXSm-22 strain significantly enhanced L-glutamic acid accumulation. These results demonstrate that the mutant-type gene which is advantageous to L-glutamic acid can be constructed even by introducing mutation in vitro at random. The sequence of the yggB gene which pL5kXSm-22 contains is shown in SEQ ID NO: 73 (22-Type Mutation).

The 22-type mutation is a mutation in which proline at position 437 is substituted with serine and a mutation in which C at position 2745 is substituted with T in SEQ ID NO: 5, for example. Further, this mutation is accompanied by a mutation in which C at position 3060 is substituted with T. This mutant-type yggB gene is shown in SEQ ID NO: 73, and the amino acid sequence of the mutant-type YggB protein encoded by this mutant-type yggB gene is shown in SEQ ID NO: 74.

[0137]

[Table 13]

<Table 13 Amount of L-glutamic Acid Produced by yggB Plasmid Subjected to Mutagenesis Treatment - 3>

Strain	OD620 (x101)	Glu (g/L)
ATCC13869ΔyggB/pL5kXS (Untreated)	0.253	0.58
ATCC13869ΔyggB/pL5kXSm22	0.232	1.10
ATCC13869ΔyggB/pL5kXSm27	0.245	0.82

[0138]

(13-3) Introduction of Mutant-Type Genes into Coryneform Bacteria, and Confirmation of L-glutamic Acid Production

The novel mutant-type yggB gene obtained in (13-2) is introduced into a coryneform bacterium. Method of introduction is as follows. The mutant-type yggB gene was introduced into pBS4S by the method stated in Example 6, and substituted with a wild-type yggB gene on the chromosome of ATCC13869. A strain in which this novel mutant-type yggB gene is introduced, and the parent ATCC13869 strain are cultured by a method similar to the method stated in Working Example 2. After completion of the culture, the amount of L-glutamic acid in the culture solution is measured by a known method to confirm that L-glutamic acid accumulation is enhanced due to the mutation-introduced strain. In this manner, the yggB gene mutant strain of which the L-glutamic acid-producing ability is enhanced can be obtained.

(14) Working Example 14

[0139]

<Sensitivity to Glutamic Acid Analogue of the Strains Carrying the Mutant-Type yggB Gene>

(14-1) Sensitivity to 4-Fluoroglutamic Acid on Solid Medium

It was predicted that strains which enhanced the L-glutamic acid-producing ability due to yggB mutation would have decreased sensitivity to L-glutamic acid analogues. Accordingly, as an L-glutamic acid analogue due to yggB mutations, a change of sensitivity to 4-fluoroglutamic acid was investigated and considered. 4-fluoroglutamic acid which had been adjusted to pH 6.7 with NaOH and sterile-filtered was added to a minimum medium stated in Working Example 4 so as to attain a final concentration of 4-fluoroglutamic acid of 7.5 mM. ATCC13869 strain, ATCC13869-L30 strain, and ATCC13869-A1 strain were spread over a CM-Dex medium and cultured overnight. Then, the bacterial cells were collected from the medium, washed with sterilized 0.85% NaCl solution, and diluted so as to attain the bacterium concentration shown in the top of

Figure 6, and spotted onto the plate. The plate was cultured at 31.5°C. The time-course is shown in Figure 6. It was shown that the ATCC13869 strain grew the fastest of three strains under the condition where 4-fluoroglutamic acid was not added. In contrast, it was shown that under the condition where 4-fluoroglutamic acid was added, the growth of the ATCC13869 strain was suppressed, and the growth of the ATCC13869-L strain and the ATCC13869-A1 strain became better than that of the ATCC13869 strain.

[0140]

(14-2) Sensitivity to 4-Fluoroglutamic Acid on Liquid Medium

4-fluoroglutamic acid was adjusted to pH 6.7 with NaOH and was sterile-filtered. This 4-fluoroglutamic acid was added to a minimum medium having the same composition as stated in Working Example 4 (except for not containing agar) so as to attain a final concentration of 1.25 mM, 2.5 mM, 5 mM, 10 mM, and 20 mM, respectively. Each of ATCC13869 strain, ATCC13869ΔyggB strain, ATCC13869-L30 strain, and ATCC13869-A1 strain was spread over a CM-Dex medium and was cultured at 31.5°C overnight. Then, the bacterial cells were collected, washed with sterilized 0.85% NaCl solution, inoculated into the liquid medium, and cultured at 31.5°C with shaking. In a medium under the condition where 4-fluoroglutamic acid is not added in each sterilized strain, when the OD660 value of the medium reached 1.0, the culture was terminated, and the obtained culture solution was diluted appropriately and spread over a CM-Dex plate. The number of colonies which appeared on the next day was measured to be a viable cell number at the end of liquid culture. Figure 7 shows the change of the relative viable cell number at each concentration of 4-fluoroglutamic acid when the cell number of the culture under the condition where 4-fluoroglutamic acid was not added was set to 1. It has been found that the ATCC13869-A1 strain and the ATCC13869-L30 strain have decreased sensitivity to 4-fluoroglutamic acid (i.e., enhanced resistance to 4-fluoroglutamic acid).

From these results, it has been shown that strains having a mutant-type yggB strain of the present invention can also be obtained by screening using sensitivity to glutamic acid structural analogues such as 4-fluoroglutamic acid.

(15) Working Example 15

[0141]

<Construction of the Mutant-Type yggB Gene-Introduced Strain in odhA Weakened Strain, and Evaluation Thereof>

[0142]

The yggB odhA-double mutant strain was constructed by using the ATCC13869-L30 strain which was constructed in Working Example 9, and the mutant-type odhA gene was

evaluated.

First, a strain in which the mutation shown in Table 14 was introduced into the odhA gene of the ATCC13869-L strain is constructed. In Table 14, nucleotide sequences of the region corresponding to nucleotide numbers 2528 to 2562 of SEQ ID NO: 43 are shown. In Table 15, amino acid sequences of the region corresponding to amino acid numbers 696 to 707 of SEQ ID NO: 44 are shown.

L30sucA8 strain into which the odhA gene having the nucleotide sequence of SEQ ID NO: 45 was introduced can be obtained as follows. The odhA gene fragment is prepared by PCR using synthetic DNAs as shown in SEQ ID NOS: 53 and 54 as primers. The obtained odhA fragment is digested with BamHI and cloned to the BamHI site of plasmid pKF19m which is attached to Mutan-Super Express Km (manufactured by Takara Bio Inc.). Then, PCR is performed using a synthetic DNA of SEQ ID NO: 55 having a phosphorylated 5'-end and a selection primer attached to Mutan-Super Express Km, and the obtained PCR product is used to transform sup0-*E. coli* strain, for example MV 1184 strain, thereby to construct a plasmid containing the mutant odhA fragment. Then, this fragment is excised with BamHI and inserted into the BamHI site of the pBS4S plasmid, to thereby construct a plasmid for introducing this mutation. The ATCC13869-L strain is transformed by a method similar to the method stated in Working Example 1, to thereby obtain a strain in which the plasmid for introducing this mutation is inserted into the chromosome. Then, a strain which is resistant to sucrose and is sensitive to kanamycin is isolated from these strains in which the plasmid is inserted into the chromosome. L30sucA8 (odhA8) strain in which the odhA gene is deleted can be constructed by further confirming the sequence of the odhA gene and selecting the strain into which an objective frame-shift was introduced.

[0143]

In addition, other odhA mutant strains can be obtained by the following methods using a yggB mutant strain.

L30sucA801 strain into which a mutant odhA gene having a nucleotide sequence of SEQ ID NO: 47 is introduced can be obtained according to the above method by using a synthetic DNA of SEQ ID NO: 56 having a phosphorylated 5'-end instead of a synthetic DNA of SEQ ID NO: 55 having a phosphorylated 5'-end.

L30sucA805 strain into which a mutant odhA gene having a nucleotide sequence of SEQ ID NO: 49 is introduced can be obtained according to the above method by using a synthetic DNA of SEQ ID NO: 57 having a phosphorylated 5'-end instead of a synthetic DNA of SEQ ID NO: 55 having a phosphorylated 5'-end.

L30sucA77 strain into which a mutant odhA gene having a nucleotide sequence of

SEQ ID NO: 51 is introduced can be obtained according to the above method by using a synthetic DNA of SEQ ID NO: 58 having a phosphorylated 5'-end instead of a synthetic DNA of SEQ ID NO: 55 having a phosphorylated 5'-end.

The L30sucA8 strain does not have an α-KGDH activity, because the odhA gene of the L30sucA8 strain has a frame-shift mutation. On the other hand, in the L30sucA801 strain, the L30sucA805 strain, and the L30sucA77 strain, these strains have mutations in which there is deletion in odhA gene but frame-shift will not occur. These strains have the α-KGDH activity, but their activity was lower than that of a non-modified strain (data not shown).

[0144]

[Table 14]

<Table 14 Partial Sequences of Mutant-type odhA Gene>

Strain Name	odhA Sequence											
ATCC13869-L	CTG	GCT	AAG	CTG	CGT	GGC	TAC	GAC	GTC	GGA	GGC	AC
L30sucA8	CTG	GCT	AAG	CTG	CGT		C	GAC	GTC	GGA	GGC	AC
sucA801	CTG	GCT	AAG	CTG	CGT		CTC	GAC	GTC	GGA	GGC	AC
sucA805	CTG	GCT	AAA	AGC	TGC		GTC	GAC	GTC	GGA	GGC	AC
sucA77	CTG	GCT	ATA	AGC	TGC		GTC	GAC	GTC	GGA	GGC	AC

[0145]

[Table 15]

<Table 15 Partial Sequences of Mutant-type α-Ketoglutarate Dehydrogenase>

Strain Name	α-KGDH Sequence											
wild	Leu	Ala	Lys	Leu	Arg	Gly	Tyr	Asp	Val	Gly	Gly	Thr
ΔsucA (L30sucA8)	Leu	Ala	Lys	Leu	Arg							
sucA801	Leu	Ala	Lys	Leu	Arg	---	Leu	Asp	Val	Gly	Gly	Thr
sucA805	Leu	Ala	Lys	Ser	Cys	---	Val	Asp	Val	Gly	Gly	Thr
sucA77	Leu	Ala	Ile	Ser	Cys	---	Val	Asp	Val	Gly	Gly	Thr

[0146]

<Culture of odhA Modified Strain, and Production of L-glutamic Acid by the odhA Modified Strain>

L-glutamic acid-producing ability of these odhA modified strains was evaluated by culturing these strains in a Sakaguchi flask. Each of the strains shown in Table 14 was cultured on a CM-Dex agar medium at 31.5°C for a whole day and night. Then, 1/6 plate

bacterial cells of this culture were inoculated to 20 ml of a flask medium (60 g/l glucose, 22.5 g/l ammonium sulfate, 1 g/l KH_2PO_4, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l $FeSO_4 \cdot 7H_2O$, 0.01 g/l $MnSO_4 \cdot 4\text{-}5H_2O$, 200 μg/l vitamin B1, 0.48 g/l soybean protein hydrolysate solution, and 300 μg/l biotin, adjusted to pH 8.0 with KOH). 1 g of calcium carbonate which had been dry heat-sterilized beforehand was added to this flask medium, and each of the strains was cultured with shaking at 115 rpm at 31.5°C. The amount of accumulated L-glutamic acid after 19 hours from the start of culture is shown in Table 16. odhA modified strains include a strain which exhibited L-glutamic acid accumulation equivalent to that of the ATCC13869-L strain. However, the sucA801 strain, the sucA805 strain, and the sucA77 strain exhibited higher L-glutamic acid accumulation than did the sucA8 strain. From these results, it has been found that the yield of L-glutamic acid is further enhanced by introducing mutations into the odhA gene in addition to the yggB gene.

[0147]

<Table 16 Amount of L-glutamic Acid Produced by odhA Modified Strain>

Strain Name	Glu (g/L)
ATCC13869-L	4.9
L30sucA8	19.8
L30sucA801	22.1
L30sucA805	23.8
L30sucA77	21.6

(16) Working Example 16

[0148]

<Construction of odhA Weakened Strain from ATCC14067 Strain and ATCC14067yggB8 Strain>

odhA-deleted strain was constructed from yggB mutant strain having 8-type mutation, and the constructed strain was cultured as well as a strain having only odhA deletion, to compare the two strains. First, a plasmid for completely deleting odhA was constructed. PCR was performed using chromosomal DNA of ATCC14067 strain as a template and synthetic DNAs shown in SEQ ID NOS: 77 and 78 to prepare an upstream side fragment of the sucA gene. Subsequently, PCR was performed using chromosomal DNA of ATCC14067 strain as a template and the synthetic DNAs shown in SEQ ID NOS: 79 and 80 to prepare a downstream side fragment of the sucA gene. Then, PCR was performed using a mixture of equimolar amounts of the upstream side fragment and the downstream side fragment as a template and synthetic DNAs of SEQ ID NOS: 81 and 82 to prepare a

gene fragment in which odhA was deleted. The obtained PCR fragment was digested with BamHI and was cloned in the pBS4S constructed in Working Example 1. The plasmid thus obtained was named pBSΔsucA47.

The obtained pBSΔsucA47 was inserted into the chromosome of ATCC14067 strain or ATCC14067yggB8 strain by a method similar to the method stated in Working Example 6, and then was removed. Then, a chromosomal DNA was prepared from the obtained kanamycin-sensitive strain. Thereafter, PCR was performed by using this chromosomal DNA as a template and the synthetic DNAs of SEQ ID NOS: 77 and 80 to select a strain in which odhA region was deleted. odhA-deleted strains thus constructed were named ATCC14067ΔodhA strain and ATCC14067ΔodhA yggB8 strain, respectively.

The constructed ATCC14067ΔodhA strain and the ATCC14067ΔodhA yggB8 strain were cultured by the method stated in Working Example 3. The results of this culture are shown in Table 17. It has been found that yggB8 mutation can enhance the yield of L-glutamic acid of the odhA mutant strain.

[0149]

[Table 17]

<Table 17 Amount of L-glutamic Acid Produced by 8-Type yggB Gene Chromosome-Introduced Strain>

	OD620 (x101)	Glu (g/L)
ATCC14067ΔodhA	0.270	5.8
ATCC14067ΔodhA yggB8	0.242	22.0

(17) Working Example 17

[0150]

<Construction of symA-Deleted Strain from 2A-1R Strain>

The symA (suppressor of ygg mutation A) gene was deleted from the 2A-1R strain having a mutation of IS insertion into yggB, which was constructed in Working Example 3, thereby to construct a strain. The obtained strain was cultured as well as the 2A-1R strain, to compare the two strains. The nucleic acid sequence and the amino acid sequence of NCgl1867 gene of the ATCC13869 strain is shown in SEQ ID NO: 86 and SEQ ID NO: 87, respectively. First, a plasmid for the purpose of completely deleting the symA gene was constructed. PCR was performed using chromosomal DNA of the ATCC13869 strain as a template and the synthetic DNAs shown in SEQ ID NOS: 88 and 89 to prepare an upstream side fragment of the symA gene. Subsequently, PCR was performed using chromosomal DNA of ATCC13869 strain as a template and the synthetic DNAs shown

in SEQ ID NOS: 90 and 91 to prepare a downstream side fragment of the symA gene. Then, PCR was performed using a mixture of equimolar amounts of the upstream side fragment and the downstream side fragment as a template and synthetic DNAs of SEQ ID NOS: 88 and 91 to prepare a gene fragment in which the symA gene was deleted. The obtained PCR fragment was digested with BglII and was cloned in the pBS4S constructed in Working Example 1. The plasmid thus obtained was named pBSΔsymA.

The obtained pBSΔsymA was inserted into the chromosome of 2A-1R strain by a method similar to the method stated in Working Example 6, and then was removed. Then, a chromosomal DNA was prepared from the obtained kanamycin-sensitive strain. Thereafter, PCR was performed by using this chromosomal DNA as a template and the synthetic DNAs of SEQ ID NOS: 88 and 91 to select a strain in which symA region was deleted. The symA-deleted strain thus constructed was named 2A-1RΔsymA strain.

The constructed 2A-1RΔsymA strain and the parent 2A-1R strain were cultured by the method stated in Working Example 3. The results of these culture are shown in Table 18. It has been found that the deletion of the symA gene can enhance the L-glutamic acid producing ability of a strain having the mutant -type yggB gene.

[0151]

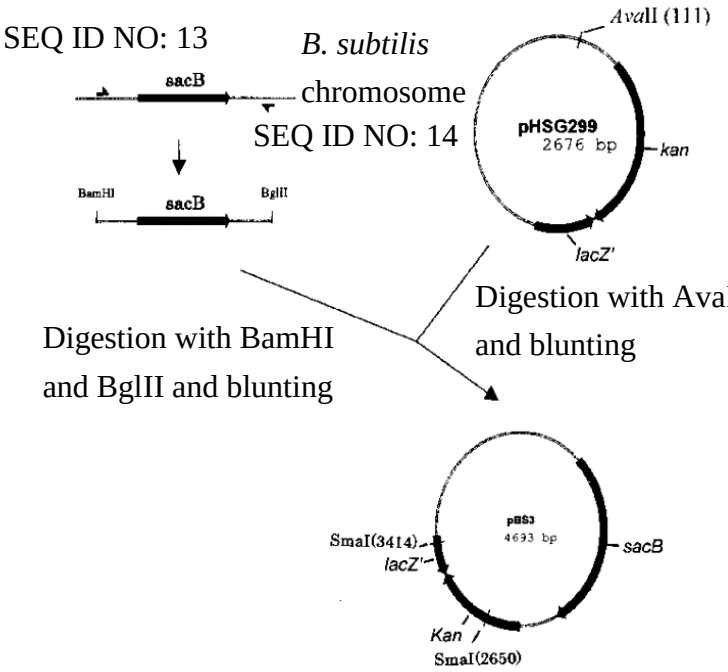
[Table 18]

<Table 18 Enhancement of L-glutamic Acid-Producing Ability by symA Gene Deletion>

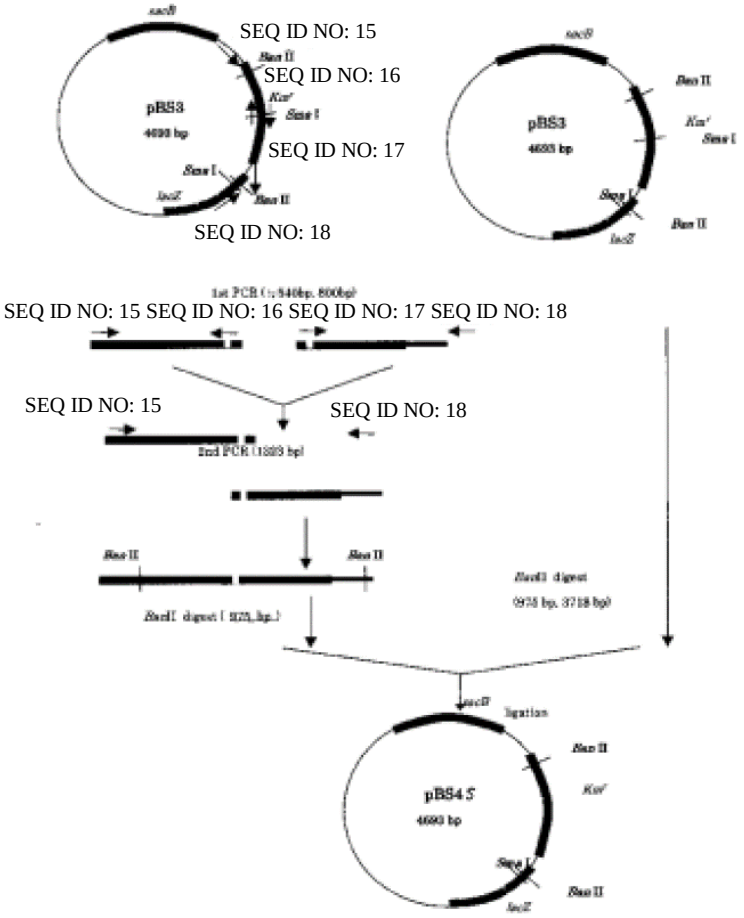
	OD620 (x51)	Glu (g/L)
2A-1R	0.846	12.4
2A-1RΔsymA	0.709	15.8

8. Drawings

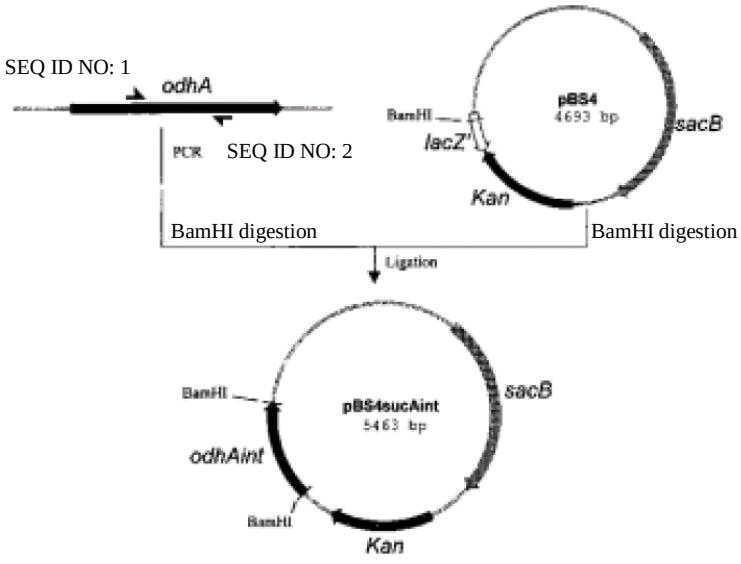
[Figure 1] A figure showing the procedure for constructing plasmid pBS3



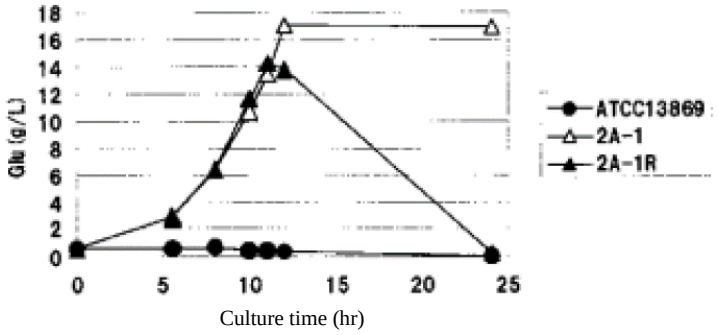
[Figure 2] A figure showing the procedure for constructing plasmid pBS4S



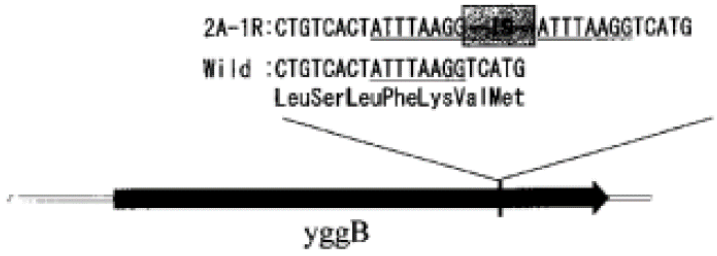
[Figure 3] A figure showing the procedure for constructing plasmid pBS4sucAint



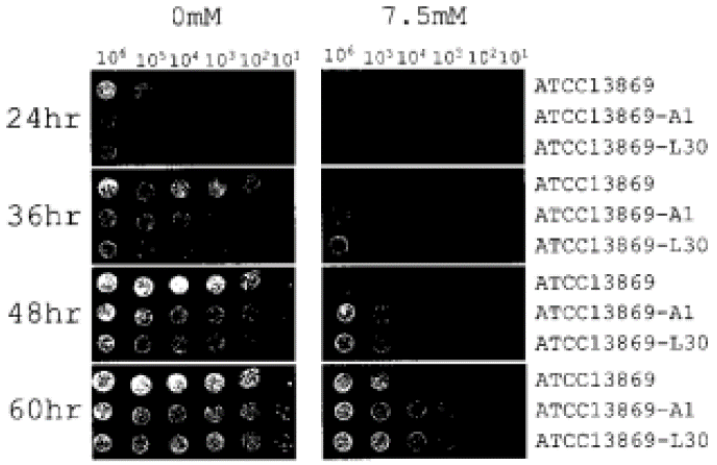
[Figure 4] A figure showing the accumulation of L-glutamic acid by the mutant-type yggB gene-introduced strain and the control strain



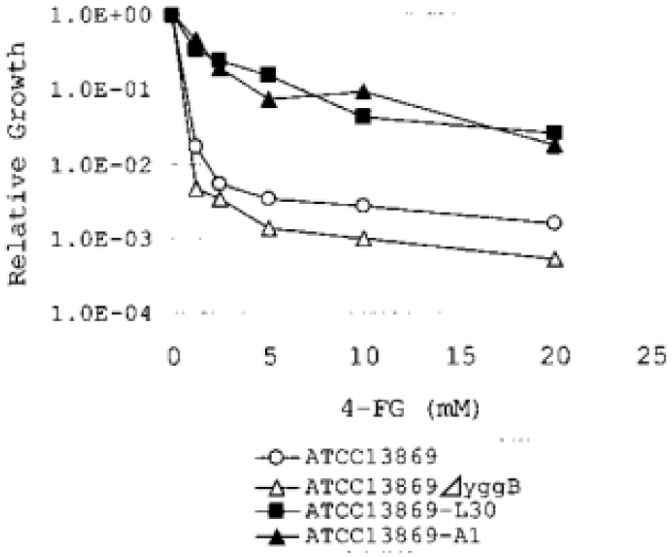
[Figure 5] A figure showing the location for inserting the mutation in a yggB gene having the 2A-1-type mutation



[Figure 6] A figure showing growth of the mutant-type yggB gene-introduced strains in a CM-Dex plate medium containing 4-fluoroglutamic acid (photograph)



[Figure 7] A figure showing growth of the mutant-type yggB gene-introduced strains in a liquid medium containing 4-fluoroglutamic acid (4-FG).



Attachment 14 "Drawings of Cited Documents"

1. Exhibit Otsu 6 Document

Fig. 1

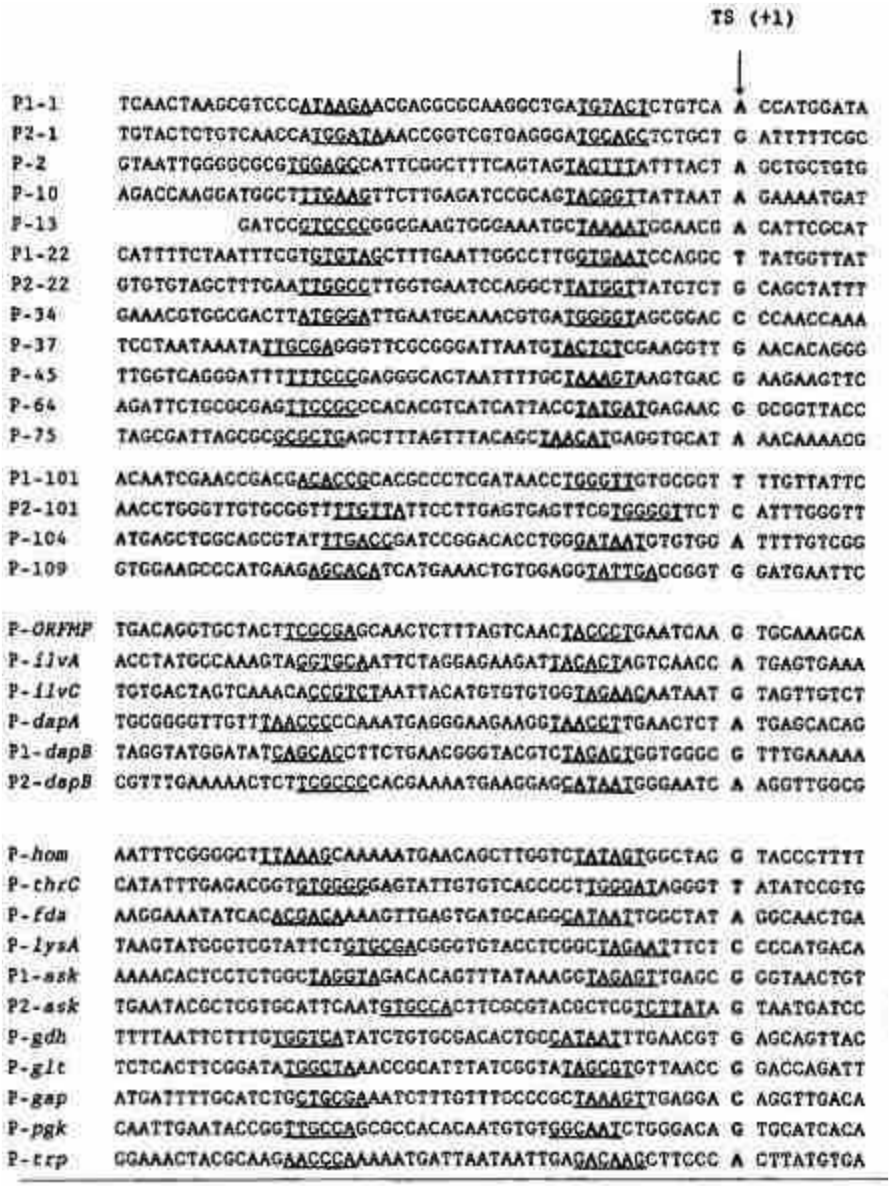


Fig. 5

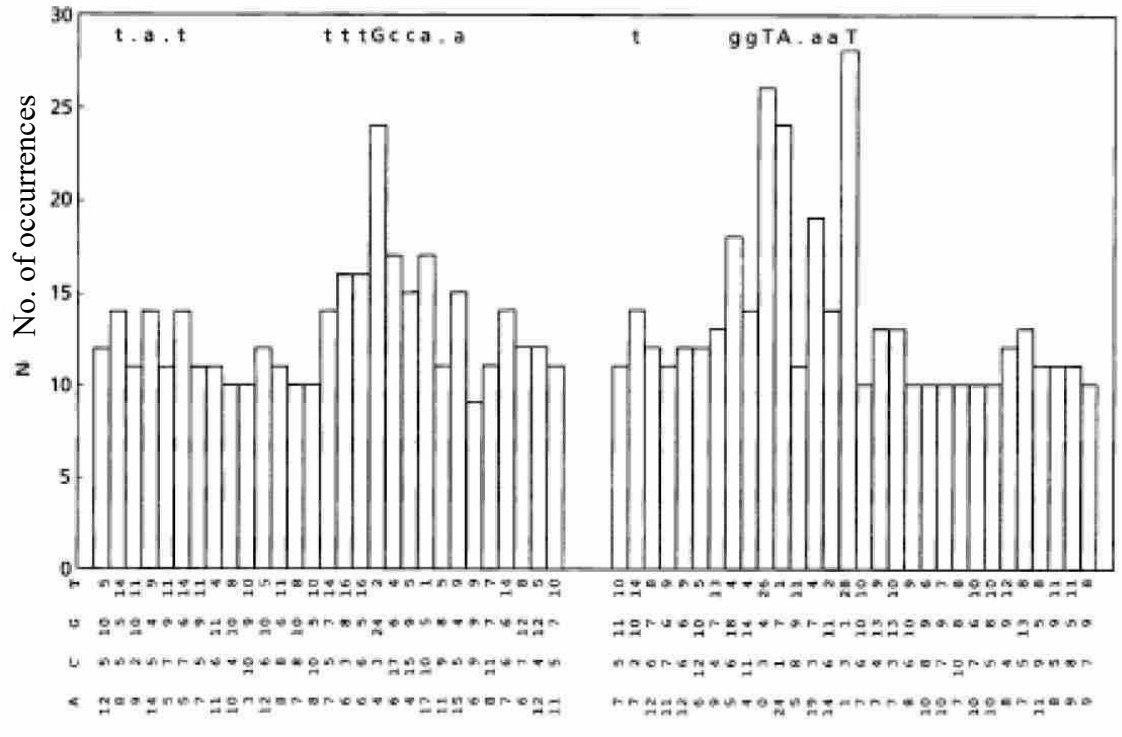


Table 2

Bacterium	Percentage conservation of single nucleotides in the consensus motif of the:											
	-35 region						-10 region					
	T	T	G	A*	C	A	T	A	T	A	A	T
C. glutamicum	48	48	73	54	45	52	79	73	33	58	42	85
E. coli	78	82	68	58	52	54	82	89	52	59	49	89
Bacillus subtilis	87	83	78	64	51	58	94	96	60	78	74	94
Lactobacillus	90	87	60	63	43	60	83	83	60	87	63	90
Streptococcus	88	76	88	59	59	47	76	10 0	65	71	65	10 0

* In the *C. glutamicum* -35 consensus sequence the fourth nucleotide is C instead of A. An A at this position was found in only 7 of the 33 promoters (21% conservation).

2. Exhibit Otsu 9 Document

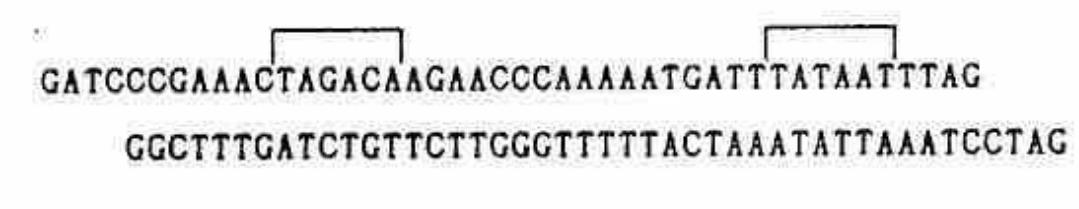
Table 9

Table 9

Strain	Bacterial cell concentration (OD)	L-glutamic acid (g/l)
ΔS	0.84	35
ΔS/pGDH	1.01	35
ΔS/pGLTA	0.83	37
ΔS/pICD	0.83	37
ΔS/pPPC	0.75	37
ΔS/pGDH+GLTA+ICD	0.95	38
ΔS/pGDH+GLTA+PPC	0.85	40
ΔS/pSAC4	0.83	35

3. Exhibit Otsu 12 Document

Nucleotide sequence of a DNA fragment shown in the upper right column on page 6



4. Exhibit Otsu 24 Document

Table 1

Efflux solute	Analytical method	Solute retained after dilution to osmolality of					
		before	1860	1060	860	710	540
		μmol・mg dm^{-1} (%)					
Gly betaine	sc. count	0.92	0.90 (98)	0.69	0.56	0.43	0.28 (30)
Proline	sc. count	0.94	0.91 (97)	0.63	0.50	0.38	0.27 (29)
Ectoine	NMR	0.52	0.51 (98)	0.50	0.46	0.44	0.29 (56)
Gly betaine[a]	NMR	0.35	-	-	0.15	0.11	0.01 (3)
+ ectoine[a]	NMR	0.45	-	-	0.34	0.32	0.23 (51)
Alanine[d]	HPLC	0.32	0.32 (100)	0.22	0.20	0.20	0.15 (47)
Glutamate[d]	enzyme	0.46	0.42 (91)	0.40	0.41	-	0.37 (80)
Lysine[d]	HPLC	0.20	0.18 (90)	0.15	0.16	0.15	0.15 (75)
ATP[b]	luciferase	0.007	0.007 (100)	0.007	0.007	0.007	0.007 (99)
Na^{+}[c,d]	flame ph.	0.57	0.22 (39)	-	0.21	0.17	0.16 (28)
K^{+}[c,d]	flame ph.	1.03	0.95 (92)	-	0.86	0.79	0.67 (65)

[a] Loading with both glycine betaine (3 mM) and ectoine (3 mM) at the same time.

[b] Efflux of ATP never exceeded 2% of the total ATP content in the cytoplasm.

[c] The case of Na^{+} and K^{+}, the steps in osmolality were 2100, 1140, 820, 420 mOsrn.

[d] Loading in the presence of 2 mM glycine betaine, in order to create a competitive situation (see text).

5. Exhibit Otsu 35 Document

Table 2

Strain	Specific activity of GDH[1)]
801[2)]	0.68
801(pAG50)	0.65
801(pAG1001)	3.71

Table 10

Strain	Specific activity of CS[1)]
801[2)]	0.50
801(pAG50)	0.52
801(pAG4001)	2.06
801(pAG4002)	2.07
801(pAG4003)	2.10

Table 11

Strain	CS[1)]	ICDH[2)]	GDH[3)]
801[4)]	0.33	0.82	0.85
801(pCI31)	1.6	3.4	0.85
801(pCG5)	1.7	0.75	3.2
801(pCIG231)	1.1	3.9	2.5

Table 13

Strain	Glutamate concentration (g/dl)[1)]	Yield based on sugar (%)[2)]
801[3)]	9.1	52
801(pAG1001)	9.2	54
801(pAG3001)	9.1	54
801(pAG4003)	9.2	53
801(pAG5001)	9.2	53
801(pIG101)	10.9	57
801(pAIG321)	10.6	58
801(pCI31)	9.1	54
801(pCG5)	9.3	56
801(pCIG231)	11.2	60
801(pCAIG4)	11.2	61

Attachment 15 "Calculation Table of the Value of Damages under Article 102, Paragraph (2) of the Patent Act for the Portion Sold by the Defendant, etc."

(omitted)

Attachment 16 "Situation of Import of Monosodium Glutamate During the Period of Use of the Defendant's Production Processes"

(omitted)

Attachment 17 "List of Products Subject to Injunction"

Monosodium glutamate with the product name "MI-POONG"
which satisfies the following requirements:
1. One produced by using *Corynebacterium glutamicum* into which a yggB gene, wherein alanine at position 100 in the amino acid sequence indicated in the sequence listing attached to this list is substituted with threonine, is introduced.
2. One produced by using *Corynebacterium glutamicum* into which a yggB gene, wherein alanine at position 98 in the amino acid sequence encoded by the yggB gene derived from *Corynebacterium callunae* (DSM20147 strain) is substituted with threonine, and valine at position 241 in that amino acid sequence is substituted with isoleucine, is introduced.

End

Sequence listing attached to Attachment 17

Met 1	Ile	Leu	Gly	Val 5	Pro	Ile	Gln	Tyr	Leu 10	Leu	Tyr	Ser	Leu	Trp 15	Asn
Trp	Ile	Val	Asp 20	Thr	Gly	Phe	Asp	Val 25	Ala	Ile	Ile	Leu	Val 30	Leu	Ala
Phe	Leu	Ile 35	Pro	Arg	Ile	Gly	Arg 40	Leu	Ala	Met	Arg	Ile 45	Ile	Lys	Gln
Arg	Val 50	Glu	Ser	Ala	Ala	Asp 55	Ala	Asp	Thr	Thr	Lys 60	Asn	Gln	Leu	Ala
Phe 65	Ala	Gly	Val	Gly	Val 70	Tyr	Ile	Ala	Gln	Ile 75	Val	Ala	Phe	Phe	Met 80
Leu	Ala	Val	Ser	Ala 85	Met	Gln	Ala	Phe	Gly 90	Phe	Ser	Leu	Ala	Gly 95	Ala
Ala	Ile	Pro	Ala 100	Thr	Ile	Ala	Ser	Ala 105	Ala	Ile	Gly	Leu	Gly 110	Ala	Gln
Ser	Ile	Val 115	Ala	Asp	Phe	Leu	Ala 120	Gly	Phe	Phe	Ile	Leu 125	Thr	Glu	Lys
Gln	Phe 130	Gly	Val	Gly	Asp	Trp 135	Val	Arg	Phe	Glu	Gly 140	Asn	Gly	Ile	Val
Val 145	Glu	Gly	Thr	Val	Ile 150	Glu	Ile	Thr	Met	Arg 155	Ala	Thr	Lys	Ile	Arg 160
Thr	Ile	Ala	Gln	Glu 165	Thr	Val	Ile	Ile	Pro 170	Asn	Ser	Thr	Ala	Lys 175	Val
Cys	Ile	Asn	Asn 180	Ser	Asn	Asn	Trp	Ser 185	Arg	Ala	Val	Val	Val 190	Ile	Pro

Ile	Pro	Met 195	Leu	Gly	Ser	Glu	Asn 200	Ile	Thr	Asp	Val	Ile 205	Ala	Arg	Ser
Glu	Ala 210	Ala	Thr	Arg	Arg	Ala 215	Leu	Gly	Gln	Glu	Lys 220	Ile	Ala	Pro	Glu
Ile 225	Leu	Gly	Glu	Leu	Asp 230	Val	His	Pro	Ala	Thr 235	Glu	Val	Thr	Pro	Pro 240
Thr	Val	Val	Gly	Met 245	Pro	Trp	Met	Val	Thr 250	Met	Arg	Phe	Leu	Val 255	Gln
Val	Thr	Ala	Gly 260	Asn	Gln	Trp	Leu	Val 265	Glu	Arg	Ala	Ile	Arg 270	Thr	Glu
Ile	Ile	Asn 275	Glu	Phe	Trp	Glu	Glu 280	Tyr	Gly	Ser	Ala	Thr 285	Thr	Thr	Ser
Gly	Thr 290	Leu	Ile	Asp	Ser	Leu 295	His	Val	Glu	His	Glu 300	Glu	Pro	Lys	Thr
Ser 305	Leu	Ile	Asp	Ala	Ser 310	Pro	Gln	Ala	Leu	Lys 315	Glu	Pro	Lys	Pro	Glu 320
Ala	Ala	Ala	Thr	Val 325	Ala	Ser	Leu	Ala	Ala 330	Ser	Ser	Asn	Asp	Asp 335	Ala
Asp	Asn	Ala	Asp 340	Ala	Ser	Ala	Ile	Asn 345	Ala	Gly	Asn	Pro	Glu 350	Lys	Glu
Leu	Asp	Ser 355	Asp	Val	Leu	Glu	Gln 360	Glu	Leu	Ser	Ser	Glu 365	Glu	Pro	Glu
Glu	Thr 370	Ala	Lys	Pro	Asp	His 375	Ser	Leu	Arg	Gly	Phe 380	Phe	Arg	Thr	Asp
Tyr 385	Tyr	Pro	Asn	Arg	Trp 390	Gln	Lys	Ile	Leu	Ser 395	Phe	Gly	Gly	Arg	Val 400

Arg Met Ser Thr Ser Leu Leu Leu Gly Ala Leu Leu Leu Leu Ser Leu
405 410 415
Phe Lys Val Met Thr Val Glu Pro Ser Glu Asn Trp Gln Asn Ser Ser
420 425 430
Gly Trp Leu Ser Pro Ser Thr Ala Thr Ser Thr Ala Val Thr Thr Ser
435 440 445
Glu Thr Ser Ala Pro Ala Ser Thr Pro Ser Met Thr Val Pro Thr Thr
450 455 460
Val Glu Glu Thr Pro Thr Met Glu Ser Ser Val Glu Thr Gln Gln Glu
465 470 475 480
Thr Ser Thr Pro Ala Thr Ala Thr Pro Gln Arg Ala Asp Thr Ile Glu
485 490 495
Pro Thr Glu Glu Ala Thr Ser Gln Glu Glu Thr Thr Ala Ser Gln Thr
500 505 510
Gln Ser Pro Ala Val Glu Ala Pro Thr Ala Val Gln Glu Thr Val Ala
515 520 525
Pro Thr Ser Thr Pro
530

End