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

[Summary]

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

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

[Best Mode for Carrying Out Invention]

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

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

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

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

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

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

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

Another exemplary embodiment of the present disclosure provides the microorganism of

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

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% $CaCO_3$, based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours.

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

[Table 5]

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

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

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

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

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

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

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% $CaCO_3$ 1 L, based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours.

[Table 6]

Strain	Putrescine (g/L)	Productivity	Fold

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

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

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

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

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

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

Each of the strains cultured therefrom in an amount of about one platinum loop was inoculated into 25 mL of titer medium (8% glucose, 0.25% soybean protein, 0.50% corn steep solids, 4% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% urea, biotin 100 g, thiamine HCl 3 mg, calcium-pantothenic acid 3 mg, nicotinamide 3 mg, 5% $CaCO_3$ based on 1 L), and cultured with shaking at 30°C at a rate of 200 rpm for 50 hours. In all cultures of strains, 1 mM

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

[Table 7]

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

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

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

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