

## ORIGINAL ARTICLE

# Gene expression of *Corynebacterium glutamicum* in response to the conditions inducing glutamate overproduction

M. Kataoka<sup>1,2</sup>, K.-I. Hashimoto<sup>2</sup>, M. Yoshida<sup>3</sup>, T. Nakamatsu<sup>2</sup>, S. Horinouchi<sup>1</sup> and H. Kawasaki<sup>2</sup>

<sup>1</sup> Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan

<sup>2</sup> Department of Environmental Materials Science, Tokyo Denki University, Tokyo, Japan

<sup>3</sup> Chemical Genetics Laboratory, RIKEN Saitama, Japan

## Keywords

amino acid, *Corynebacterium glutamicum*, gene expression, glutamate overproduction, transcriptome analysis.

## Correspondence

H. Kawasaki, Department of Environmental Materials Science, Tokyo Denki University, 2-2, Nishiki-cho, Kanda, Chiyoda-ku, Tokyo 101-8457, Japan. E-mail: kawasaki@cck.dendai.ac.jp

2005/0834: received 25 July 2005, revised 4 November 2005 and accepted 28 November 2005

doi:10.1111/j.1472-765X.2006.01905.x

## Abstract

**Aim:** The ultimate aim is to elucidate the molecular mechanisms for glutamate overproduction by *Corynebacterium glutamicum*.

**Methods and Results:** Gene expression in response to the conditions inducing glutamate overproduction was investigated by using a DNA microarray technique. Most genes involved in the EMP pathway, the PPP, and the TCA cycle were downregulated, while five genes that were highly upregulated (*NCgl0917*, *NCgl2944*, *NCgl2945*, *NCgl2946*, and *NCgl2975*) were identified under all the three conditions for overproduction that are studied here. Gene products of *NCgl2944*, *NCgl2945*, and *NCgl2946* were highly homologous to each other, did not resemble any other protein, and have remained uncharacterized thus far. The product of *NCgl0917* showed a similarity to a few hypothetical and uncharacterized proteins. *NCgl2975* was homologous to metal-binding proteins. **Conclusions:** The decrease in the activity of 2-oxoglutarate dehydrogenase complex, a key enzyme that is downregulated during glutamate overproduction, can be mainly attributed to the downregulation of *odhA* and *sucB*. Five highly upregulated genes were also identified.

**Significance and Impact of the Study:** Although fermentative production of glutamate has been carried out for more than 45 years, information on the molecular mechanisms of glutamate overproduction is still limited. This study further elucidates these mechanisms.

## Introduction

*Corynebacterium glutamicum* is an aerobic, gram-positive, nonsporulating bacterium that has been widely used for the fermentative production of glutamate and a number of other amino acids for more than 45 years (Hermann 2003). The history of *C. glutamicum* as an amino acid producer dates back to 1956 when the bacterium was first isolated (Kinoshita *et al.* 1957; Udaka 1960). This discovery was an epoch-making event because it was believed that primary metabolites, including amino acids, were never accumulated in micro-organisms. This was a turning point and currently, various microorganisms industrially produce many primary metabolites. Of the amino

acids that are fermentatively produced, glutamate is the most prominent, and its production as a sodium salt is 1.5 million tonnes per year.

Glutamate overproduction in *C. glutamicum* is induced by biotin limitation (Shiio *et al.* 1962); by specific detergents, such as polyoxyethylene sorbitan monopalmitate (Tween 40, Takinami *et al.* 1965); and by a sublethal concentration of penicillin (Numheimer *et al.* 1970). A recent study showed that the activity of 2-oxoglutarate dehydrogenase complex (ODHC) is greatly reduced under all the aforementioned conditions (Shingu and Terui 1971; Kawahara *et al.* 1997), which leads to an increase in the carbon flow towards the synthesis of glutamate at the ODHC branch point (Shimizu *et al.* 2003).

Recently, *dtsR1* was cloned to function as a multicopy suppressor gene that could overcome the hypersensitivity of a mutant strain to Tween 40 (Kimura *et al.* 1997a). The amino acid sequence of DtsR1 showed homology to that of a subunit of acyl-coenzyme A (CoA) carboxylases of various origins, and disruption of *dtsR1* resulted in fatty acid auxotrophy. Hence, DtsR1 is presumed to represent a subunit of acetyl-CoA carboxylase (Kimura *et al.* 1997b), which catalyses the first step in fatty acid biosynthesis. On the basis of studies on *dtsR1*, it is assumed that a decrease in the DtsR1 cellular concentration due to biotin limitation and Tween 40 addition triggers a reduction in ODHC activity and leads to glutamate overproduction (Kimura *et al.* 1999). However, it was observed that penicillin treatment did not decrease the DtsR1 cellular concentration, but reduced the ODHC activity (Kimura *et al.* 1999). The reason for the identical response of the ODHC activity to treatments with the two agents having different sites of primary action remains unclear (Eggeling *et al.* 2001).

As mentioned earlier, the information on the molecular mechanism for glutamate overproduction is still limited, although fermentative production of glutamate has been carried out for more than 45 years, and enormous efforts have been devoted to clarify the mechanism of glutamate overproduction. As the first step for understanding the molecular mechanisms for glutamate overproduction by *C. glutamicum*, we determined the gene expression under conditions of glutamate overproduction by using a DNA microarray technique.

## Materials and methods

### Chemicals and enzymes

Tween 40 and Tween 80 (polyoxyethylene sorbitan mono-oleate) were purchased from Wako Pure Chemical Industries (Osaka, Japan); glutamate oxidase, from Yamasa (Chiba, Japan); and penicillin G, from Merck (NJ, USA).

### Strains and culture conditions

*C. glutamicum* ATCC13869, a wild-type strain, was grown at 31.5°C for 24 h on CM2B agar (Miwa *et al.* 1985). The cells were collected from the surface of the agar and inoculated in a 500-ml Sakaguchi flask that contained 20 ml of the medium for glutamate production (330 mM glucose, 230 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 7 mM KH<sub>2</sub>PO<sub>4</sub>, 2 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 40 µM FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.7 µM thiamine, 120 nM D-biotin, 0.48 g of nitrogen per litre soybean protein hydrolysate and 50 g l<sup>-1</sup> CaCO<sub>3</sub>). After 24 h of cultivation at 31.5°C, 2 ml of the culture was inoculated

into 20 ml of fresh medium in a 500-ml Sakaguchi flask. For the biotin-limited conditions, the medium contained 12 nM D-biotin. After 3 h of cultivation at 31.5°C, Tween 40, Tween 80, or penicillin G was added to the medium to achieve final concentrations of 1.5 g l<sup>-1</sup>, 5 g l<sup>-1</sup>, or 2 200 U l<sup>-1</sup>, respectively.

### Determination of amount of glutamate

The amount of glutamate was determined enzymatically by using glutamate oxidase, which produces hydrogen peroxide, 2-oxoglutaric acid, and ammonia from glutamate (Kusakabe *et al.* 1983).

### Transcriptome analysis

Transcriptome analysis was performed according to a previously described method (Ulijasz *et al.* 2004). Total RNA was extracted with RNeasy (Qiagen, Crawley, UK). cDNA was synthesized from the total RNA by using random hexamer primers and Superscript II reverse transcriptase (Invitrogen, CA, USA). The cDNA was partially digested by DNase I, followed by 3'-end labelling with Biotin-N6-ddATP (PerkinElmer, MA, USA) by deoxynucleotidyl terminal transferase. A DNA microarray with 32 types of specific 24-mer probes for each gene (Nimblegen Systems, WI, USA) was used. After hybridization at 42°C for 16 h, by using streptavidin and the biotinylated anti-streptavidin antibody amplification was performed, and finally, a Cy3-streptavidin fluoro-dye was conjugated. Array scanning, data extraction, and calibration were performed by using the Nimblegen Systems. Array normalization was performed by the Robust Multi-chip Analysis (Bolstad *et al.* 2003). The genes that showed a statistically significant change in transcription in comparison with the control were extracted using the Dunnett method ( $\alpha = 0.05$ , Dunnett 1955). Clustering analysis was performed using UPGMA (coefficient of distance: Pearson correlation).

## Results

As the first step for understanding the molecular mechanisms of glutamate overproduction by *C. glutamicum*, we determined the gene expression under conditions of glutamate overproduction by using a DNA microarray technique. For comparison, the gene expression in response to the addition of Tween 80, which does not induce glutamate overproduction, was also analysed as a negative control.

Glutamate was accumulated in our experiments under the conditions described in "Materials and methods" at concentrations of 160 mM under the biotin-limited con-

dition, at 71 mM in the presence of Tween 40, and at 150 mM in the presence of penicillin (Fig. 1). Total RNA was extracted from cells grown for 12 h, when the rate of glutamate production was the highest under the three different conditions though the rates were fairly steady, and a significant amount of glutamate had accumulated (Fig. 1).

The results from the DNA microarray analysis are summarized in Table 1. We observed that most genes involved in the biosynthetic pathway of glutamate, including the EMP pathway, the PPP, and the TCA cycle, tended to be downregulated, as compared with the control (cultivated in a biotin-rich medium). The genes involved in the PPP were downregulated to a lesser extent as compared with those involved in the EMP pathway and the TCA cycle. The genes involved in the anaplerotic pathway for the TCA cycle were also downregulated. The genes involved in the glyoxylate cycle remained almost unaltered, except for one of the two isocitrate lyase genes.

ODHC is a key enzyme located at the branching point in the metabolic pathway towards succinyl-CoA synthesis (TCA cycle) and glutamate synthesis. This enzyme is composed of three subunits, namely, 2-oxoglutarate dehydrogenase (E1 $\alpha$ ; EC 1.2.4.2), dihydrolipoamide S-succinyltransferase (E2 $\alpha$ ; EC 2.3.1.61), and dihydrolipoamide dehydrogenase (E3; EC 1.8.1.4). Decreased ODHC activity during the overproduction of glutamate by *C. glutamicum* has been suggested to be critical for glutamate overproduction (Shingu and Terui 1971; Kawahara *et al.* 1997). Of the genes encoding the ODHC subunits, *odhA* (Usuda *et al.* 1996) encoding E1 $\alpha$  and *sucB* encoding E2 $\alpha$  were strongly repressed during transcription; however, *lpd* (Schwinde *et al.* 2001) encoding E3 was not repressed (Table 1). Therefore, the decrease in ODHC activity may

be attributed mainly to the downregulation of *odhA* and *sucB*.

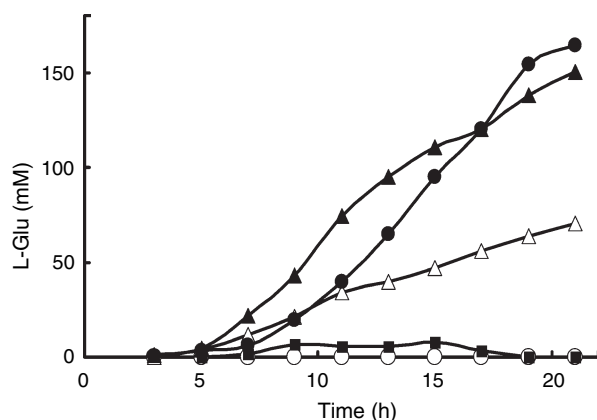
The expression of both *dtsR1* and *accBC*, which were suggested to encode the subunits of acetyl-CoA carboxylase (Kimura *et al.* 1997b; Jaeger *et al.* 1996) and to be involved in glutamate overproduction, was downregulated in the presence of Tween 40 and penicillin. On the other hand, the biotin-limited condition exerted almost no effect on the *dtsR1* transcription, but it severely downregulated the transcription of *accBC* (Table 1). *C. glutamicum* has three *dtsR1* homologues – *dtsR2* (*accD2*), *accD3*, and *accD4* (Gande *et al.* 2004) – in addition to *dtsR1* (*accD1*). Unlike the transcription of *dtsR1*, the transcription of *dtsR2* was affected only slightly, and those of *accD3* and *accD4* were almost unaltered (Table 1).

The transcriptome analysis also revealed that the transcription of the glutamate dehydrogenase gene was unaltered under all the conditions of glutamate overproduction (Table 1). This is in accordance with the observation that the activity of glutamate dehydrogenase is unaltered under all the conditions, inducing glutamate overproduction (Kawahara *et al.* 1997).

The genes that were highly upregulated (>eightfold) under at least one condition that induced glutamate overproduction were extracted, and these are listed in Table 2. All the genes that are listed were also upregulated under all the other conditions, suggesting the critical involvement of these genes in glutamate overproduction. However, their functions or their roles in glutamate overproduction were unknown. PSI-BLAST searches showed that the gene products of *NCgl2944*, *NCgl2945*, and *NCgl2946* were highly homologous to each other and resembled no other protein in the database; additionally these remain uncharacterized. The *NCgl0917* product showed similarity to the hypothetical and uncharacterized proteins of *C. glutamicum*, *NCgl2252*, and proteins of a few other bacteria. *NCgl2975* was homologous to metal-binding proteins.

## Discussion

Most genes involved in the biosynthetic pathway of glutamate, were downregulated. This suggested that glutamate overproduction is mainly attributed to the pre-existing enzymes rather than to newly synthesized enzymes. The genes involved in the anaplerotic pathway of the TCA cycle were also downregulated; a slight effect on the pyruvate carboxylase gene and a severe reduction of the phosphoenolpyruvate carboxykinase gene expression accounted for the efficient production of oxaloacetate, which, along with acetyl-CoA, is a substrate for glutamate biosynthesis. The genes involved in the glyoxylate cycle were almost unaltered, except for one of the



**Figure 1** L-Glutamate accumulation. (○) control; (●) biotin limitation; (△) Tween 40 addition; (▲) penicillin addition; (■) Tween 80 addition.

**Table 1** Transcriptional response of genes encoding enzymes involved in the biosynthetic pathway of glutamate from glucose and of genes possibly involved in glutamate overproduction to the conditions which induce glutamate overproduction

Enzyme (annotation)	NCBI accession number	Fold change			
		Biotin limitation	Tween 40 addition	Penicillin addition	Tween 80 addition
<b>EMP pathway</b>					
Glucose-6-phosphate isomerase	NCgl0817	-5.0	-1.7	-5.6	-1.7
6-Phosphofruktokinase	NCgl1202	-1.5	1.1	-2.3	2.3
	NCgl1860	-1.4	1.0	-1.6	1.0
Fructose-bisphosphate aldolase	NCgl2673	-15.3	-4.8	-24.7	-3.1
Triosephosphate isomerase	NCgl1524	-5.9	-1.7	-8.3	-1.6
Glyceraldehyde-3-phosphate dehydrogenase	NCgl1526	-4.5	-2.5	-15.9	-1.9
	NCgl0900	-1.2	1.0	-1.5	1.0
3-Phosphoglycerate kinase	NCgl1525	-9.3	-2.4	-16.1	-1.8
Phosphoglycerate mutase	NCgl0390	-2.1	-1.4	-9.7	-1.1
	NCgl0423	-1.3	1.0	-1.6	1.0
	NCgl1013	1.1	1.0	1.0	1.0
	NCgl2268	-1.1	1.3	-1.1	1.2
Enolase	NCgl0935	-6.2	-2.1	-13.3	-1.8
Pyruvate kinase	NCgl2008	-3.8	-1.9	-11.4	1.2
<b>PPP</b>					
Glucose-6-phosphate dehydrogenase	NCgl1514	-1.3	-1.1	-1.3	-1.1
6-Phosphogluconolactonase	NCgl1516	-1.2	1.0	-1.3	1.0
6-Phosphogluconate dehydrogenase	NCgl1396	-1.9	-1.4	-3.6	-1.4
Ribose 5-phosphate isomerase	NCgl2337	-1.6	-1.1	-1.6	1.1
Ribulose-phosphate 3-epimerase	NCgl1536	-2.6	-1.7	-2.9	-1.4
Transketolase	NCgl1512	-4.9	-2.9	-5.8	-1.6
Transaldolase	NCgl1513	-4.9	-3.1	-5.0	-1.9
<b>TCA cycle</b>					
Pyruvate dehydrogenase (E1)	NCgl2167	-2.0	-1.1	-15.1	-2.0
Pyruvate dehydrogenase (E3)	NCgl0355	-1.1	1.0	-1.1	1.0
	NCgl0658	-1.6	-1.3	-1.7	-1.3
Citrate synthase	NCgl0630	1.1	1.2	1.1	1.8
	NCgl0795	-5.6	-3.8	-6.4	-1.8
Aconitase	NCgl1482	-7.5	-6.5	-20.1	-3.9
Isocitrate dehydrogenase	NCgl0634	-4.8	-1.8	-6.4	-1.5
2-Oxoglutarate dehydrogenases (E1)	NCgl1084	-9.9	-3.9	-12.9	-2.7
2-Oxoglutarate dehydrogenase (E2)	NCgl2126	-6.3	-2.8	-7.3	-2.2
2-Oxoglutarate dehydrogenase (E3)	NCgl0355	-1.1	1.0	-1.1	1.0
2-Oxoglutarate dehydrogenase (E3)	NCgl0658	-1.6	-1.3	-1.7	-1.3
Succinyl-CoA synthetase (alpha)	NCgl2476	1.0	1.0	1.0	1.0
Succinyl-CoA synthetase (beta)	NCgl2477	1.1	1.1	1.1	1.0
Succinate dehydrogenase (cytochrome)	NCgl0359	-35.0	-12.7	-41.8	-3.7
Succinate dehydrogenase (flavoprotein)	NCgl0360	-25.9	-13.8	-40.1	-3.9
Succinate dehydrogenase (iron-sulfur protein)	NCgl0361	-16.3	-7.2	-21.5	-2.4
Fumarate hydratase	NCgl0967	-10.8	-3.6	-13.3	-2.7
Malate dehydrogenase	NCgl2297	-9.3	-2.7	-23.0	-3.2
<b>Glyoxylate cycle</b>					
Isocitrate lyase	NCgl0096	-1.1	1.2	-1.2	1.0
	NCgl2248	-2.8	-1.9	4.3	2.2
Malate synthase	NCgl2247	-1.2	-1.2	1.9	1.2
<b>TCA anaplerotic pathway</b>					
Pyruvate carboxylase	NCgl0659	-1.6	-1.5	-1.8	-1.2
Pyruvate carboxylase	NCgl1273	-2.2	-1.6	-2.5	-1.6
Phosphoenolpyruvate carboxylase	NCgl1523	-3.2	-2.3	-7.0	-1.8
Phosphoenolpyruvate carboxykinase	NCgl2765	-9.0	-2.7	-15.9	-5.0
Malic enzyme	NCgl2904	1.0	1.0	-1.2	1.0

**Table 1** (contd)

Enzyme (annotation)	NCBI accession number	Fold change			
		Biotin limitation	Tween 40 addition	Penicillin addition	Tween 80 addition
Others					
Glutamate dehydrogenase	NCgl1999	-1.5	1.0	-1.8	1.0
DtsR1 (AccD1)	NCgl0678	1.1	-2.3	-3.5	-2.0
DtsR2 (AccD2)	NCgl0677	-1.8	1.0	-2.4	1.0
AccD3	NCgl2772	-1.2	1.0	-1.3	1.0
AccD4	NCgl0797	-1.1	1.0	-1.3	1.0
AccBC	NCgl0670	-3.4	-2.3	-2.2	-2.0

**Table 2** Genes which were strongly up-regulated (>8-fold) under at least one condition that induced glutamate overproduction

NCBI accession number	Fold change			
	Biotin limitation	Tween 40 addition	Penicillin addition	Tween 80 addition
NCgl0917	11.3	5.4	2.7	1.6
NCgl2944	1.9	8.0	3.4	1.7
NCgl2945	7.2	25.7	29.8	2.1
NCgl2946	4.2	27.3	8.5	2.0
NCgl2975	1.5	3.6	10.4	-1.2

two isocitrate lyase genes, suggesting that no regulation by acetyl-CoA had occurred.

The effect of Tween 40 on the gene expression was less than that of biotin limitation or penicillin addition, reflecting on the lower glutamate production in the presence of Tween 40 than under the other conditions in our experiments.

The expression of both *dtsR1* and *accBC*, which were suggested to encode the subunits of acetyl-CoA carboxylase (Kimura *et al.* 1997b; Jaeger *et al.* 1996) and to be involved in glutamate overproduction, was downregulated in the presence of Tween 40 and penicillin. On the other hand, the biotin-limited condition had negligible effects on *dtsR1* transcription, but severely downregulated the transcription of *accBC* (Table 1). This implies that *dtsR1* encoding the enzyme subunit responsible for fatty acid synthesis was not downregulated by fatty acid starvation, as a result of biotin limitation, whereas the transcription of *accBC* encoding the biotin-binding subunit was downregulated by biotin limitation. These results do not appear to be in agreement with the hypothesis (Kimura *et al.* 1999) that DtsR1 regulates ODHC activity by direct interaction with ODHC under all the conditions inducing glutamate overproduction. However, the protein concentrations in the cell do not always correlate with transcription; therefore, further investigations would be necessary to clarify this point.

The transcription of *dtsR2* was affected only slightly and those of *accD3* and *accD4* were almost unaltered (Table 2). This is consistent with the finding that those are involved in other processes, such as mycolic acid biosynthesis (Gande *et al.* 2004). Therefore, we assume that *dtsR2*, *accD3*, and *accD4* are not involved in glutamate overproduction.

Clustering analysis of genes that showed more than 1.5-fold change under all the conditions by UPGMA revealed that *NCgl0917*, *NCgl2944*, *NCgl2945*, and *NCgl2946* were in the same cluster defined as highly upregulated genes under all the conditions inducing glutamate overproduction and consisted of six genes, namely, *NCgl0226*, *NCgl0917*, *NCgl2841*, *NCgl2944*, *NCgl2945*, and *NCgl2946*. This also suggests the possible involvement of genes listed in Table 2 in glutamate overproduction. Since the characteristics of the other five clusters were not common among the conditions inducing glutamate overproduction, discussions of these is beyond the scope of this manuscript.

During the preparation of this manuscript, Radmacher *et al.* (2005) reported the transcriptome analysis of glutamate-overproducing *C. glutamicum* elicited by ethambutol, an inhibitor of cell wall biosynthesis. They found that 18 genes were highly upregulated by ethambutol, two of which, *NCgl2944* and *NCgl2946*, were identical to the genes detected in the present study.

Although extensive further investigations are required to elucidate the molecular mechanisms of glutamate overproduction by *C. glutamicum*, the results of the gene expression analysis reported in this paper are the first step. The study of the upregulation and downregulation of genes under the conditions inducing glutamate overproduction will reveal a possible network of gene expression.

## References

- Bolstad, B., Irizarry, R., Astrand, M. and Speed, T. (2003) A comparison of normalization methods for high density

- oligonucleotide array data based on bias and variance. *Bioinformatics* **19**, 185–193.
- Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. *J Am Stat Assoc* **50**, 1096–1121.
- Eggeling, L., Krumbach, K. and Sahm, H. (2001) L-Glutamate efflux with *Corynebacterium glutamicum*: why is penicillin treatment or Tween addition doing the same? *J Mol Microbiol Biotechnol* **3**, 67–68.
- Gande, R., Gibson, K.J.C., Brown, A.K., Krumbach, K., Dover, L.G., Sahm, H., Shioyama, S., Oikawa, T *et al.* (2004) Acyl-CoA carboxylases (*accD2* and *accD3*), together with a unique polyketide synthase (*Cg-pks*), are key to mycolic acid biosynthesis in *Corynebacteriaceae* such as *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*. *J Biol Chem* **279**, 4847–4857.
- Hermann, T. (2003) Industrial production of amino acids by coryneform bacteria. *J Biotechnol* **104**, 155–172.
- Jaeger, W., Peters-Wendisch, P.G., Kalinowski, J. and Puhler, A. (1996) A *Corynebacterium glutamicum* gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins. *Arch Microbiol* **166**, 76–82.
- Kawahara, Y., Takahashi-Fuke, K., Shimizu, E., Nakamatsu, T. and Nakamori, S. (1997) Relationship between the glutamate production and the activity of 2-oxoglutarate dehydrogenase in *Brevibacterium lactofermentum*. *Biosci Biotechnol Biochem* **61**, 1109–1112.
- Kimura, E., Abe, C., Kawahara, Y. and Nakamatsu, T. (1997a) Molecular cloning of a novel gene, *dtsR*, which rescues the detergent sensitivity of a mutant derived from *Brevibacterium lactofermentum*. *Biosci Biotechnol Biochem* **60**, 1565–1570.
- Kimura, E., Abe, C., Kawahara, Y. and Nakamatsu, T. and Tokuda, H. (1997b) A *dtsR* gene-disrupted mutant of *Brevibacterium lactofermentum* requires fatty acids for growth and efficiently produces L-glutamate in the presence of an excess of biotin. *Biochem Biophys Res Commun* **234**, 157–161.
- Kimura, E., Yagoshi, C., Kawahara, Y., Ohsumi, T., Nakamatsu, T. and Tokuda, H. (1999) Glutamate overproduction in *Corynebacterium glutamicum* triggered by a decrease in the level of a complex comprising DtsR and biotin-containing subunit. *Biosci Biotechnol Biochem* **63**, 1274–1278.
- Kinoshita, S., Udaka, S. and Shimono, M. (1957) Studies on the amino acid fermentation. Part I. Production of L-glutamic acid by various microorganisms. *J Gen Appl Microbiol* **3**, 193–205.
- Kusakabe, H., Midorikawa, Y., Fujishima, T., Kuninaka, A. and Yoshino, H. (1983) Purification and properties of new enzyme, L-glutamate oxidase, from *Streptomyces* sp. X-119-6 grown on wheat bran. *Agric Biol Chem* **47**, 1323–1382.
- Miwa, K., Matsui, K., Terabe, M., Ito, K., Ishida, M., Takagi, H., Nakamori, S. and Sano, K. (1985) Construction of novel shuttle vectors and a cosmid vector for the glutamic acid-producing bacteria *Brevibacterium lactofermentum* and *Corynebacterium glutamicum*. *Gene* **39**, 281–296.
- Numheimer, T.D., Birnbaum, J., Ihnen, E. and Demain, A.L. (1970) Product inhibition of the fermentative formation of glutamic acid. *Appl Microbiol* **20**, 215–217.
- Radmacher, E., Stansen, K.C., Bersa, G.S., Alderwick, L.J., Maughan, W.N., Hollweg, G., Sahm, H., Wendisch, V.F. *et al.* (2005) Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. *Microbiology* **151**, 1359–1368.
- Schwinde, J.W., Hertz, P.F., Sahm, H., Eikmanns, B.J. and Guyonvarch, A. (2001) Lipoamide dehydrogenase from *Corynebacterium glutamicum*: molecular and physiological analysis of the *lpd* gene and characterization of the enzyme. *Microbiology* **147**, 2223–2231.
- Shiio, I, Otsuka, S. and Takahashi, M. (1962) Effect of biotin on the bacterial formation of glutamic acid: Glutamate formation and cellular permeability of amino acids. *J Biochem* **51**, 56–62.
- Shimizu, H., Tanaka, H., Nakato, A., Nagahisa, K., Kimura, E. and Shioya, S. (2003) Effects of the changes in enzyme activities on metabolic flux redistribution around the 2-oxoglutarate branch in glutamate production by *Corynebacterium glutamicum*. *Bioprocess Biosyst Eng* **25**, 291–298.
- Shingu, H. and Terui, G. (1971) Studies on the process of glutamic acid fermentation at the enzyme level: I. On the changes of  $\alpha$ -ketoglutaric acid dehydrogenase in the course of culture. *J Ferment Technol* **49**, 400–405.
- Takinami, K., Yoshi, H., Tsuru, H. and Okada, H. (1965) Biochemical effects of fatty acid and its derivatives on L-glutamic acid fermentation: Part III. Biotin-Tween 60 relationship in accumulation of L-glutamic acid and the growth of *Brevibacterium lactofermentum*. *Agric Biol Chem* **29**, 351–359.
- Udaka, S. (1960) Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. *J Bacteriol* **79**, 754–755.
- Ulijasz, A.T., Andes, D.R., Glasner, J.D. and Weisblum, B. (2004) Regulation of iron transport in *Streptococcus pneumoniae* by RitR, an orphan response regulator. *J Bacteriol* **186**, 8123–8136.
- Usuda, Y., Tsujimoto, N., Abe, C., Asakura, Y., Kimura, E., Kawahara, Y., Kurahashi, O. and Matsui, H. (1996) Molecular cloning of the *Corynebacterium glutamicum* (*Brevibacterium lactofermentum*' AJ12036) *odhA* gene encoding a novel type of 2-oxoglutarate dehydrogenase. *Microbiology* **142**, 3347–3354.