J. Microbiol. Biotechnol. (2004), 14(2), 373–378

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Regulation of Enzymes Involved in Methionine Biosynthesis in *Corynebacterium glutamicum*

YEOM, HYE-JIN[1], BYUNG-JOON HWANG[1], MYONG-SOK LEE[2], YOUNHEE KIM[3], AND HEUNG-SHICK LEE[1,4]*

[1]*Graduate School of Biotechnology, Korea University, Anam-Dong, Sungbuk-Ku, Seoul 136-701, Korea*
[2]*Department of Biological Science, Sookmyung Women's University, Seoul 140-740, Korea*
[3]*Department of Oriental Medicine, Semyung University, Checheon, Chungbuk 390-230, Korea*
[4]*Department of Biotechnology, Korea University, Jochiwon, Choongnam 339-700, Korea*

Received: November 11, 2003
Accepted: December 11, 2003

Abstract The regulatory mechanism of methionine biosynthesis in *Corynebacterium glutamicum* was analyzed at the protein and gene expression level. *O*-Acetylhomoserine sulfhydraylase (encoded by *metY*) was inhibited by 10 mM methionine to a residual activity of 10% level, whereas no such inhibition was found with cystathionine γ-synthase (encoded by *metB*) and cystathionine β-lyase (encoded by *metC*). The enzymatic activity of homoserine acetyltransferase (encoded by *metX*) was repressed to a residual activity of 25% level by 10 mM methionine which was added to the growth medium. Cystathionine γ-synthase and cystathionine β-lyase were also repressed by 10 mM methionine, but only to a residual activity of 50–70% level. *O*-Acetylhomoserine sulfhydrylase was very sensitive to repression by 10 mM methionine, showing residual activity of 13%. In addition, homoserine acetyltransferase was also repressed by 10 mM cysteine to 50% of its original activity. No repression of the enzymes by *S*-adenosyl methionine was observed. The pattern of repression by methionine indicated that the *metB* and *aecD* genes might be regulated by a common mechanism, while the *metA* and *metY* genes are differently regulated.

Key words: *Corynebacterium glutamicum*, methionine, homoserine acetyltransferase, cystathionine γ-synthase, cystathionine β-lyase

Corynebacterium glutamicum is a Gram-positive non-sporulating organism and has been widely used for the industrial production of amino acids. Due to the role of the organism in amino acid production, biosynthetic pathways leading to lysine and other industrially important amino acids have been studied in detail [3, 8, 11, 16, 27, 28].

The biosynthetic pathways leading to methionine have been studied in diverse organisms and show similarities as well as differences. In *C. glutamicum*, the first step, acylation of homoserine to acetyl homoserine, is catalyzed by homoserine acetyl transferase (EC 2.3.1.31), encoded by *metX* [21]. Subsequent formation of homocysteine from *O*-acetylhomoserine can be accomplished through two different routes: a transsulfuration pathway via cystathionine utilizes cysteine as the sulfur donor, and a direct sulfhydrylation pathway utilizes sulfide as the sulfur donor (Fig. 1). In the transsulfuration pathway, *O*-acetylhomoserine is converted to cystathionine by cystathionine γ-synthase (EC 4.2.99.9), the product of *metB* [9]. In the reaction, cysteine serves as the sulfur donor. In the transsulfuration pathway, cystathionine is further hydrolyzed to homocysteine, pyruvate, and ammonia by cystathionine β-lyase (EC 4.4.1.8) encoded by *aecD* (also known as *metC*) [17, 25]. In addition, *C. glutamicum* can directly synthesize homocysteine from *O*-acetylhomoserine, a pathway known as the direct sulfhydrylation pathway. The pathway bypasses cystathionine in the transsulfuration pathway and is catalyzed by *O*-acetylhomoserine sulfhydrylase (EC 4.2.99.10) encoded by *metY* [10]. The MetY enzyme utilizes sulfide as the sulfur donor. Because of the presence of parallel pathways, a single deletion of *metB*, *metC*, or *metY* does not result in methionine auxotrophy [10, 17, 25, 26, 33]. However, double deletion of *metB* and *metY* (or *aecD* and *metY*) results in methionine auxotrophy [10, 26]. The *metY* gene of *C. glutamicum* encoding *O*-acetylhomoserine sulfhydrylase is located immediately upstream of *metX*, separated by 143 bp, however, the *metX* and *metY* genes are independently expressed [10]. The final step, the methylation of homocysteine

*Corresponding author
Phone: 82-41-860-1415; Fax: 82-41-864-2665;
E-mail: hlee@korea.ac.kr

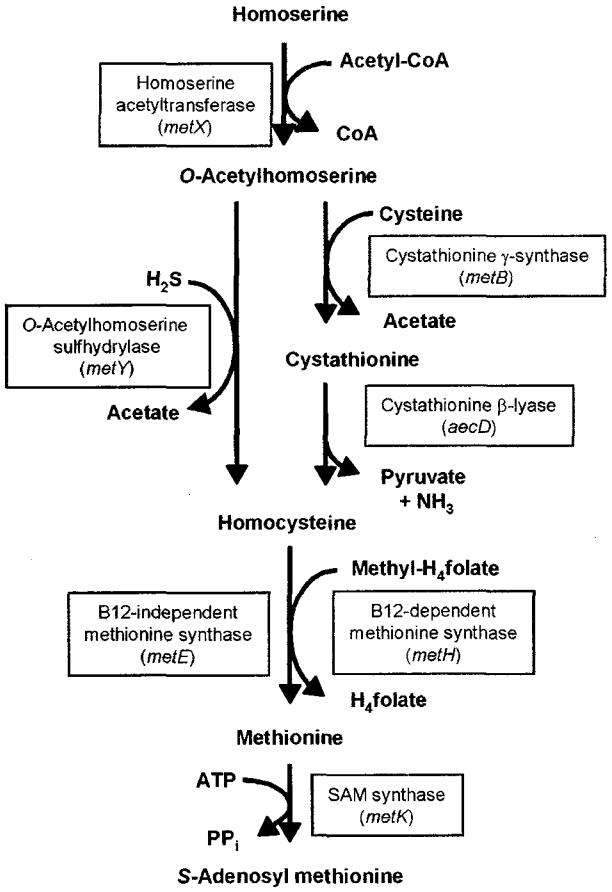

Fig. 1. Methionine biosynthetic pathway in *C. glutamicum*. Abbreviation: SAM, *S*-adenosyl methionine.

to methionine, is catalyzed by methionine synthase. In *C. glutamicum*, two forms of methionine synthase are present, which are encoded by *metE* and *meH*, respectively [26].

In recent years, some genes involved in methionine biosynthesis in *C. glutamicum* have been isolated or identified. In addition, Rey *et al.* [24] isolated a transcriptional regulator McbR from *C. glutamicum* for genes involved in sulfur-containing amino acids. Apparently, it functions as a transcriptional repressor by acting on several genes involved in the biosynthesis of amino acids methionine and cysteine. In a *mcbR* mutant strain, genes for methionine biosynthesis, such as *metY* and *metK*, and genes for sulfur assimilation and cysteine biosynthesis are derepressed. Since only a few *met* genes are repressed by the McbR protein, there must be additional regulators for other *met* genes of *C. glutamicum*.

In this study, the expression of methionine biosynthetic enzymes was analyzed and reexamined to gain insight on how methionine biosynthesis in *C. glutamicum* is regulated. Based on biochemical evidences, a regulatory mechanism of methionine biosynthesis in *C. glutamicum* is proposed.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions

C. glutamicum AS019E12 [5] cells were cultured at 37°C in LB [29] and at 30°C in MB [5], respectively. Minimal medium for *C. glutamicum* was MCGC [32]. Glucose was added to the final concentration of 1%. Sulfur-free minimal medium (pH 7.4) for *C. glutamicum* was composed of 1% glucose, 50 mM Na_2HPO_4, 25 mM KH_2PO_4, 20 mM NaCl, 30 mM NH_4Cl, 2 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.1 mM $FeCl_2$, 0.01 mM $MnCl_2$, and 1 mg/l biotin.

Biochemical Analysis

Cell extracts were prepared as described previously [12]. Cells were harvested by centrifugation and washed with 100 mM Tris-HCl (pH 7.5), containing 20 mM KCl, 33 mM $MgCl_2$, 10 mM $MnCl_2$, and 5% glycerol, and resuspended in 5 ml of the same buffer. Cells were disrupted by shaking vigorously with glass beads (212-300 microns, Sigma, U.S.A.) in a mini-bead beater (Biospec Products, U.S.A.), and the cell debris was removed by centrifugation for 30 min at 15,000 ×*g*. The supernatant was used as crude extract.

Homoserine acetyltransferase was assayed at room temperature in 1 ml assay mixture containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.2 mM acetyl coenzyme A, 2 mM homoserine, 0.5 mM DTNB [5,5'-dithio-bis(2-nitrobenzoic acid)], and appropriate amounts of the enzyme. Optical changes were monitored at 412 nm. Cystathionine γ-synthase was assayed using the method of Ravanel *et al.* [23]. The assay mixture of 0.1 ml, containing 20 mM MOPS-NaOH (pH 7.5), 1 mM cysteine, 10 mM *O*-acetylhomoserine, 1 mM dithiothreitol, and appropriate amounts of enzyme, was incubated at 30°C for 10 min. The reaction was stopped by adding 50 µl of 20% trichloroacetic acid (TCA), the precipitated proteins were removed by centrifugation for 5 min at 15,000 ×*g*, and the activity of cystathionine γ-synthase present in the supernatant was assayed by measuring disappearance of the cysteine: Fifty µl of the TCA-treated reaction mixture was added to a glass tube containing 100 µl of concentrated acetic acid and 200 µl of ninhydrin solution (2.5 g dissolved in 100 ml mixture containing 60 ml of concentrated acetic acid and 40 ml of concentrated HCl). The above mixture was boiled for 10 min and added to 650 µl of ice-cold 95% ethanol. The amount of cysteine was determined by measuring absorbance at 560 nm (the molar extinction coefficient of cysteine = 25,000 $M^{-1}cm^{-1}$). Cystathionine β-lyase was assayed as follows. The assay mixture contained 100 mM Tris-HCl (pH 8.5), 0.1 mM NADH, 1 mM *L*-cystathionine, 5 units of lactate dehydrogenase, and appropriate amounts of crude extract. Optical changes were monitored at 340 nm. Assay of *O*-acetylhomoserine sulfhydrylase was performed by the method of Foglino *et al.* [4]. The reaction mixture

was composed of 100 mM Tris (pH 8.0), 10 mM *O*-acetylhomoserine, 10 mM Na_2S, 0.2 mM pyridoxal 5'-phosphate, and enzyme in the final volume of 100 μl. *O*-acetylhomoserine was synthesized by the method of Nagai and Flavin [19]. The reaction was started by addition of Na_2S, incubated at 30°C for 10 or 30 min, and then stopped by adding 100 μl of 30% TCA. After removing the precipitated protein by centrifugation (15,000 ×*g*, 5 min), the content of homocysteine in supernatant was measured using nitroprusside: In a glass tube, 100 μl of H_2O, 100 μl of TCA-stopped reaction mixture, 600 μl of saturated NaCl, 100 μl of 1.5 M Na_2CO_3 (containing 67 mM KCN), and 100 μl of 2% nitroprusside were added in the given order. Homocysteine was quantified by measuring absorbance at 520 nm. A 0.1 μmol of homocysteine in 0.1 ml of TCA-stopped reaction mixture yielded an A_{520} of 0.74.

Protein was measured by the method of Bradford [1], with bovine serum albumin as the standard.

RESULTS

Growth-Associated Activities of Methionine Biosynthetic Enzymes

The expression of methionine biosynthetic enzymes was monitored during growth. For the experiment, *C. glutamicum* cells were grown in glucose minimal medium, and samples were taken at appropriate time intervals for the measurement of enzyme activities. As shown in Fig. 2, methionine biosynthetic enzymes, including homoserine acetyltransferase (*metX*), cystathionine γ-synthase (*metB*), cystathionine β-lyase (*aecD*), and *O*-acetyl homoserine sulfhydrylase (*metY*), showed their maximal activity at the early stationary phase. In particular, the specific activity of homoserine acetyltransferase (*metX*) and cystathionine β-lyase (*aecD*) was higher than those of others. Based on the data,

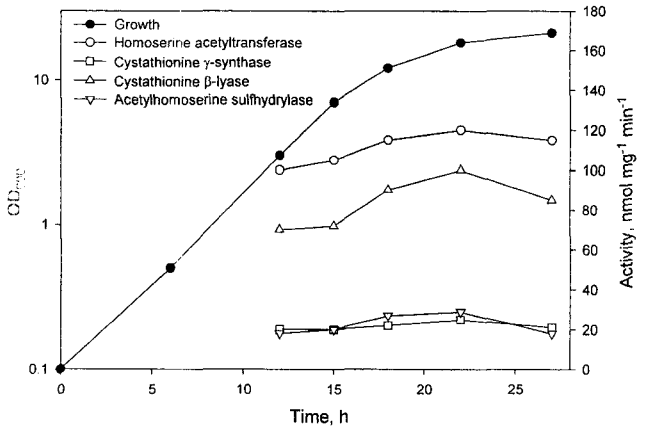

Fig. 2. Activities of *C. glutamicum* enzymes involved in methionine biosynthesis during growth.

Table 1. Inhibition of enzymes involved in methionine biosynthesis.[a]

Amino acids added	Specific activity, nmol min^{-1} mg^{-1}			
	CGS[b]	CBL	OAHSH	HK
None	27	166	28	780
Methionine	28	122	8	690
Threonine	28	148	26	300
Lysine	25	164	28	720
Isoleucine	36	206	26	700
Methionine, threonine	27	113	9	290
Methionine, threonine, lysine	26	119	9	210

[a]The enzymes were induced by growth of *C. glutamicum* cells to the early stationary phase on MCGC minimal medium containing 1% glucose. Cells were harvested, disrupted, and assayed for the activity in the presence of 10 mM amino acids.
[b]CGS, cystathionine γ-synthase; CBL, cystathionine β-lyase; OASH, *O*-acetyl homserine sulfhydrylase; HK, homoserine kinase.

therefore, subsequent experiments on enzyme assays were carried out with cells grown to the early stationary phase.

Analysis for Feedback Inhibition

The effects of various amino acids on the activity of methionine biosynthetic enzymes were analyzed with crude extracts prepared from cells grown in glucose minimal medium to the early stationary phase. For the experiment, 10 mM amino acids were added to the assay mixture, and the effect on enzyme activity was monitored. As shown in Table 1, the activities for cystathione γ-synthase and cystathionine β-lyase were not affected by aspartate family amino acids which were added alone or in combination to the assay mixture. However, the enzyme activity of *O*-acetylhomoserine sulfhydrylase was inhibited by methionine. Adding 10 mM methionine to the assay mixture resulted in 70% reduction in the activity. Other amino acids, such as threonine, lysine, and isoleucing, showed no effect on the activity. As previously reported, homoserine kinase was inhibited by L-threonine.

Analysis for Repression

The effects of various amino acids on the expression of methionine biosynthetic enzymes were analyzed. Crude extracts were prepared from the cells grown on minimal medium containing 10 mM each amino acids. As shown in Table 2, methionine repressed homoserine acetyltransferase, cystathionine γ-synthase, cystathionine β-lyase, and *O*-acetylhomserine sulfhydrylase. Compared to the others, the repression of homoserine acetyltransferase (25% residual activity) and *O*-acetyl homserine sulfhydrylase (13% residual activity) was much greater. Repression of cystathionine γ-synthase (50% residual activity) and cystathionine β-lyase (67% residual activity) by methionine was relatively weak. For all four enzymes, no apparent inhibition by lysine, threonine, and isoleucine was observed.

Table 2. Repression of enzymes involved in methionine biosynthesis.[a]

Amino acids added	Specific activity, nmol min^{-1} mg^{-1}				
	HAT[b]	CGS	CBL	OAHSH	HK
None	120	30	104	15	800
Methionine	30	14	70	2	200
Threonine	130	42	130	13	790
Lysine	130	36	94	12	720
Isoleucine	110	36	102	12	690
Methionine/threonine	30	13	72	5	190
Methionine/threonine/lysine	30	12	66	1	170

[a]The enzymes were induced by growth of *C. glutamicum* cells to the early stationary phase on MCGC minimal medium containing 1% glucose and 10 mM amino acids. Cells were harvested, disrupted, and assayed for the activity as described in the Materials and Methods.
[b]HAT, homoserine acetyltransferase; CGS, cystathionine γ-synthase; CBL, cystathionine β-lyase; OASH, *O*-acetyl homserine sulfhydrylase; HK, homoserine kinase.

In order to obtain further understanding of the regulation of methionine biosynthetic enzymes, repression was tested under various concentrations of methionine. As shown in Table 3, 5 mM methionine achieved maximal repression (25% residual activity) for homoserine acetyltransferase. Compared to homoserine acetyltransferase, repression of cystationine γ-synthase and cystathionine β-lyase by L-methionine appeared to be more sensitive, achieving maximal inhibition at a lower concentration of methionine, although the degree of repression was less. As shown by Hwang *et al.* [10], *O*-acetylhomserine sulfhydrylase was very sensitive to repression by methionine, showing almost complete repression at 0.5 mM. These data suggest that the *metB* and *aecD* genes may be regulated by a common mechanism, while *metA* is differently regulated. It appears that the expression of *metY* is also regulated by a different mechanism. Recently, Rey *et al.* [24] reported a putative repressor McbR for the *metY* gene. In accordance with the present data, the expression of *metA*, *metB*, and *metC* were not affected in a *mcbR* mutant strain.

DISCUSSION

In this study, the expression of methionine biosynthetic enzymes in *C. glutamicum* was analyzed. Although it has been known that methionine represses the expression of homoserine acetyltransferase (*metX*), cystathionine γ-synthase (*metB*), cystathionine β-lyase (*aecD*), and *O*-acetyl homoserine sulfhydrylase (*metY*) [10, 13, 14, 15, 18, 20], no simultaneous analyses of these enzymes have been carried out. As shown in this study, methionine biosynthesis in mucroorganisms is highly regulated by repression and inhibition. The inhibition of *O*-acetyl homoserine sulfhydrylase by methionine in *C. glutamicum* (50% inhibition by 10 mM methionine), as observed by Ozaki and Shiio [20], was confirmed in the present study, although the degree of inhibition was different; i.e. 72% inhibition by 10 mM methionine in this study. However, under the condition where homoserine acetyltransferase is highly inhibited by S-adenosyl methionine [20], the inhibition of *O*-acetyl homoserine sulfhydrylase by methionine may not physiologically be meaningful.

The different pattern of repression of *metX*, *metB*, *aecD*, and *metY* may indicate involvement of different kinds of regulators for each gene. The coordinate regulation of *metB* and *aecD* may indicate that the genes are regulated by a common repressor. In the same sense, the *metX* and *metY* genes are also regulated by different kinds of regulators. In accordance with the above contention, Rey *et al.* [24] isolated a transcriptional regulator McbR from *C. glutamicum* for genes involved in sulfur-containing amino acids. Apparently, it functions as a transcriptional repressor by acting on several genes involved in the biosynthesis of amino acids, methionine and cysteine. Among the methionine biosynthetic genes, only the expression of *metY* and *metK* were affected by the *mcbR* gene.

In organisms utilizing both transsulfuration and direct sulfhydrylation pathways, such as *Schizosaccharomyces pombe*, *N. crassa*, *Aspergillus nidulans*, and higher plants, the direct sulfhydrylation pathway may play a minor role

Table 3. Repression of enzymes involved in methionine biosynthesis.[a]

Amino acids	Concentration, mM	Specific activity, nmol min^{-1} mg^{-1}			
		HAT[b]	CGS	CBL	OAHSH
None		100	28	120	16
Methionine	0.1	57	16	57	4.3
	0.5	38	14	58	2.7
	1	30	14	50	2.2
	5	25	13	55	2.0
	10	27	11	47	1.9
S-Adenosyl methionine	0.5	96	27	96	16
Cysteine	10	57	26	145	15

[a]The enzymes were induced by growth of *C. glutamicum* cells to the early stationary phase on MCGC minimal medium containing 1% glucose and 10 mM amino acids. Cells were harvested, disrupted, and assayed for the activity as described in the Materials and Methods.
[b]HAT, homoserine acetyltransferase; CGS, cystathionine γ-synthase; CBL, cystathionine β-lyase; OAHSH, *O*-acetyl homserine sulfhydrylase.

in methionine biosynthesis [2, 7, 22, 34, 35]. As demonstrated by radioisotopic studies, the majority of homocysteine sulfur atoms are derived from cysteine via transsulfuration in the organisms. However, in *P. aeruginaos* and *P. putida,* the direct sulfhydrylation pathway appears to be favored over transsulfuration [31]. In the organism, cysteine is not converted to methionine via cystathionine and homocysteine under most growth conditions. The enzymes catalyzing the transsufuration pathway, such as cystathionine γ-synthase and cystathionine β-lyase, are only expressed when cysteine is supplied as a sole sulfur source. The direct sulfhydrylation pathway of *C. glutamicum* may also play a distinct role. Genetic and biochemical data suggest that *C. glutamicum* utilizes both transsulfuration and direct sulfhydrylation pathways with almost equal efficiency [10]. In addition, the *metY* gene of *C. glutamicum* is efficiently expressed. The stronger repression of *metY* than that of *metB* by methionine may suggest a distinctive role for the direct sulfhydrylation pathway. By analogy with the biosynthetic pathways for lysine in *C. glutamicum* [30], which utilize either succinylase or dehydrogenase pathways depending on the availability of ammonium ions, one may speculate a similar mechanism for the methionine biosynthetic pathways in terms of sulfur availability.

Acknowledgments

This work was supported by grants from BASF Korea (to H.-S. Lee) and the Ministry of Science and Technology (via 21C Microbial Genomics and Applications Center to H.-S. Lee).

REFERENCES

1. Bradford, M. M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.
2. Bright, S. W. J., P. J. Lea, and B. J. Miflin. 1979. The regulation of methionine biosynthesis and metabolism in plants and bacteria. *Ciba Found. Symp.* **72:** 101-117.
3. de Graaf, A. A., L. Eggeling, and H. Sahm. 2001. Metabolic engineering for L-lysine production by *Corynebacterium glutamicum*. *Adv. Biochem. Eng. Biotechnol.* **73:** 9--29.
4. Foglino, M., F. Borne, M. Bally, G. Ball, and J. C. Patte. 1995. A direct sulfhydrylation pathway is used for methionine biosynthesis in *Pseudomonas aeruginosa*. *Microbiology* **141:** 431-439.
5. Follettie, M. T., O. P. Peoples, C. Agoropoulou, and A. J. Sinskey. 1993. Gene structure and expression of the *Corynebacterium flavum* N13 *ask-asd* operon. *J. Bacteriol.* **175:** 4096-4103.
6. Follettie, M. T. and A. J. Sinskey. 1986. Recombinant DNA technology for *Corynebacterium glutamicum*. *Food Technol.* **40:** 88-94.
7. Giovanelli, J., S. H. Mudd, and A. H. Datko. 1978. Homocysteine biosynthesis in green plants. Physiological importance of the transsulfuration pathway in *Chlorella sorokiniana* growing under steady state conditions with limiting sulfate. *J. Biol. Chem.* **253:** 5665-5677.
8. Herrman, T. 2003. Industrial production of amino acids by coryneform bacteria. *J. Biotechnol.* **104:** 155-172.
9. Hwang, B.-J., Y. Kim, H.-B. Kim, H.-J. Hwang, J.-H. Kim, and H.-S. Lee. 1999. Analysis of *Corynebacterium glutamicum* methionine biosynthetic pathway: Isolation and analysis of *metB* encoding cystathionine γ-synthase. *Mol. Cells* **9:** 300-308.
10. Hwang B.-J., H.-J. Yeom, Y. Kim, and H.-S. Lee 2002. *Corynebacterium glutamicum* utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis. *J. Bacteriol.* **184:** 1277-1286.
11. Jetten, M. S. M. and A. J. Sinskey. 1993. Characterization of phosphoenolpyruvate carboxykinase from *Corynebacterium glutamicum*. *FEMS Microbiol. Lett.* **111:** 183-188.
12. Jetten, M. S. and A. J. Sinskey. 1995. Recent advances in the physiology and genetics of amino acid-producing bacteria. *Crit. Rev. Biotechnol.* **15:** 73-103.
13. Kase, H. and K. Nakayama. 1974. Production of *O*-acetyl-L-homoserine by methionine analog-resistant mutants and regulation of homoserine-*O*-transacetylase in *Corynebacterium glutamicum*. *Agric. Biol. Chem.* **38:** 2021-2030.
14. Kase, H. and K. Nakayama. 1974b. The regulation of L-methionine synthesis and the properties of cystathionine γ-synthase and β-cystathionase in *Corynebacterium glutamicum*. *Agric. Biol. Chem.* **38:** 2235-2242.
15. Kase, H. and K. Nakayama. 1975. Isolation and characterization of *S*-adenosylmethionine-requiring mutants and role of *S*-adenosylmethionine in the regulation of methionine biosynthesis in *Corynebacterium glutamicum*. *Agric. Biol. Chem.* **39:** 161-168.
16. Kim, H.-J., J.-S. Park, H.-S. Lee, and Y. Kim. 2002. Utilization of *lacZ* to isolate regulatory genes from *Corynebacterium glutamicum*. *J. Microbiol. Biotechnol.* **12:** 336-339.
17. Kim, J.-W., H.-J. Kim, Y. Kim, M.-S. Lee, and H.-S. Lee. 2001. Properties of the *Corynebacterium glutamicum metC* gene encoding cystathionine β-lyase. *Mol. Cells* **11:** 220-225.
18. Lee, J., A. Goel, M. M. Ataai, and M. M. Domach. 2002. Flux regulation patterns and energy audit of *E. coli* B/r and K-12. *J. Microbiol. Biotechnol.* **12:** 258-267.
19. Nagai, S. and M. Flavin. 1971. Synthesis of *O*-acetylhomoserine. *Methods Enzymol.* **17B:** 423-424.
20. Ozaki, H. and I. Shiio. 1982. Methionine biosynthesis in *Brevibacterium flavum*: Properties and essential role of *O*-acetylhomoserine sulfhydrylase. *J. Biochem.* **91:** 1163-1171.
21. Park, S.-D., J.-Y. Lee, Y. Kim, J.-H. Kim, and H.-S. Lee. 1998. Isolation and analysis of *metA*, a methionine biosynthetic gene encoding homoserine acetyltransferase in *Corynebacterium glutamicum*. *Mol. Cells* **8:** 286-294.

22. Paszewski, A., W. Prazmo, J. Nadolska, and M. Regulski. 1984. Mutations affecting the sulphur assimilation pathway in *Aspergillus nidulans*: Their effect on sulphur amino acid metabolism. *J. Gen. Microbiol.* **130:** 1113-1121.
23. Ravanel, S., M. Droux, and R. Douce. 1995. Methionine biosynthesis in higher plants. I. Purification and characterization of cystathionine γ-synthase from spinach chloroplasts. *Arch. Biochem. Biophys.* **316:** 572-584.
24. Rey D. A., A. Pühler, and J. Kalinowski. 2003. The putative transcriptional repressor McbR, member of the TetR-family, is involved in the regulation of the metabolic metwork directing the synthesis of sulfur containing amino acids in *Corynebacterium glutamicum*. *J. Biotechnol.* **103:** 51-65.
25. Rossol, I. and A. Pühler. 1992. The *Corynebacterium glutamicum aecD* gene encodes a C-S lyase with α,β-elimination activity that degrades aminoethylcysteine. *J. Bacteriol.* **174:** 2968-2977.
26. Rückert, C., A. Pühler, and J. Kalinowski. 2003. Genome-wide analysis of the L-methionine biosynthetic pathway in *Corynebacterium glutamicum* by targeted gene deletion and homologous complementation. *J. Biotechnol.* **104:** 213-228.
27. Sahm, H., L. Eggeling, and A. A. de Graaf. 2000. Pathway analysis and metabolic engineering in *Corynebacterium glutamicum*. *Biol. Chem.* **381:** 899-910.
28. Sahm, H., L. Eggeling, B. Eikmanns, and R. Krämer. 1995. Metabolic design in amino acid producing bacterium *Corynebacterium glutamicum*. *FEMS Microbiol. Rev.* **16:** 243-252.
29. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
30. Schrumpf, B., A. Schwarzer, J. Kalinowski, A. Pühler, L. Eggeling, and H. Sahm. 1991. A functionally split pathway for lysine synthesis in *Corynebacterium glutamicum*. *J. Bacteriol.* **173:** 4510-4516.
31. Vermeij, P. and M. A. Kertesz. 1999. Pathways of assimilative sulfur metabolism in *Pseudomonas putida*. *J. Bacteriol.* **181:** 5833-5837.
32. von der Osten, C. H., C. Gioannetti, and A. J. Sinskey. 1989. Design of a defined medium for growth of *Corynebacterium glutamicum* in which citrate facilitates iron uptake. *Biotechnol. Lett.* **11:** 11-16.
33. Wada, M., N. Awano, K. Haisa, H. Takagi, and S. Nakamori. 2002. Purification, characterization and identification of cysteine desulfhydrase of *Corynebacterium glutamicum*, and its relationship to cysteine production. *FEMS Microbiol. Lett.* **217:** 103-107.
34. Wiebers, J. L. and H. R. Garner. 1967. Homocysteine and cysteine synthetases of *Neurospora crassa*. Purification, properties, and feedback control of activity. *J. Biol. Chem.* **242:** 12-23.
35. Yamagata, S. 1989. Roles of *O*-acetyl-L-homoserine sulfhydrylases in microorganisms. *Biochimie* **71:** 1125-1143.