JOURNAL OF BACTERIOLOGY, Mar. 2002, p. 1277–1286 Vol. 184, No. 5
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.5.1277–1286.2002

Corynebacterium glutamicum Utilizes both Transsulfuration and Direct Sulfhydrylation Pathways for Methionine Biosynthesis

Byung-Joon Hwang,[1] Hye-Jin Yeom,[1] Younhee Kim,[2] and Heung-Shick Lee[1]*

Graduate School of Biotechnology, Korea University, Anam-Dong, Sungbuk-Ku, Seoul 136-701,[1] and Department of Oriental Medicine, Semyung University, Checheon, Chungbuk 390-230,[2] Korea

Received 20 August 2001/Accepted 27 November 2001

A direct sulfhydrylation pathway for methionine biosynthesis in *Corynebacterium glutamicum* was found. The pathway was catalyzed by *metY* encoding *O*-acetylhomoserine sulfhydrylase. The gene *metY*, located immediately upstream of *metA*, was found to encode a protein of 437 amino acids with a deduced molecular mass of 46,751 Da. In accordance with DNA and protein sequence data, the introduction of *metY* into *C. glutamicum* resulted in the accumulation of a 47-kDa protein in the cells and a 30-fold increase in *O*-acetylhomoserine sulfhydrylase activity, showing the efficient expression of the cloned gene. Although disruption of the *metB* gene, which encodes cystathionine γ-synthase catalyzing the transsulfuration pathway of methionine biosynthesis, or the *metY* gene was not enough to lead to methionine auxotrophy, an additional mutation in the *metY* or the *metB* gene resulted in methionine auxotrophy. The growth pattern of the *metY* mutant strain was identical to that of the *metB* mutant strain, suggesting that both methionine biosynthetic pathways function equally well. In addition, an *Escherichia coli metB* mutant could be complemented by transformation of the strain with a DNA fragment carrying corynebacterial *metY* and *metA* genes. These data clearly show that *C. glutamicum* utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis. Although *metY* and *metA* are in close proximity to one another, separated by 143 bp on the chromosome, deletion analysis suggests that they are expressed independently. As with *metA*, methionine could also repress the expression of *metY*. The repression was also observed with *metB*, but the degree of repression was more severe with *metY*, which shows almost complete repression at 0.5 mM methionine in minimal medium. The data suggest a physiologically distinctive role of the direct sulfhydrylation pathway in *C. glutamicum*.

Corynebacterium glutamicum is a gram-positive nonsporulating 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 (16, 20, 23, 32).

The biosynthetic pathways leading to methionine have been studied in diverse organisms and show similarities as well as differences (Fig. 1) (47). The first step, acylation of homoserine catalyzed by homoserine acetyl (or succinyl)-transferase (the product of *metA*), is common to all of the organisms, even though the source of the transferred acyl group is different. While the enzyme uses succinyl coenzyme A (succinyl-CoA) as the substrate in several prokaryotes such as enteric bacteria (3, 10, 38, 41), it uses acetyl-CoA in many other organisms, such as *Saccharomyces cerevisiae* (42), *Neurospora crassa* (18), *Aspergillus nidulans* (25), *Leptospira meyeri* (4), *Brevibacterium flavum* (26), and *C. glutamicum* (17, 29). As a result, the product of the step is either acetylhomoserine or succinylhomoserine, depending on the organism. Formation of homocysteine from acylhomoserine can occur in two different ways (Fig. 1). The transsulfuration pathway via cystathionine utilizes cysteine as the sulfur donor, while the direct sulfhydrylation pathway utilizes inorganic sulfur assimilated from sulfate. *Escherichia coli* uses the transsulfuration pathway which is catalyzed by cystathionine γ-synthase (the product of *metB*) and cystathionine β-lyase (the product of *metC*) (38). Organisms such as *S. cerevisiae* (6, 42), *Rhizobium etli* (41), *Pseudomonas aeruginosa* (10), and *L. meyeri* (2) utilize the direct sulfhydrylation pathway catalyzed by acylhomoserine sulfhydrylase (the product of *metY* or *metZ*). The last step, formation of methionine from homocysteine, is catalyzed by homocysteine methyltransferase encoded by *metE* (or *metH*). Although yeast, fungi, and green plants were reported to have functional transsulfuration and direct sulfhydrylation pathways (8, 13, 25, 42), no prokaryote has been clearly shown to have both pathways. Unlike the closely related *Brevibacterium flavum*, which may use only the direct sulfhydrylation pathway for methionine biosynthesis (28), enzyme activities of the transsulfuration pathway have been detected in the extracts of *C. glutamicum* cells, and the pathway has been shown to be functional for methionine biosynthesis in the organism (14, 17, 19).

Even though some genes involved in methionine biosynthesis in *C. glutamicum* were isolated in recent years, information on the biosynthesis of methionine in the organism is still very limited, and the biosynthetic pathways have not been clarified yet. We recently isolated the *metA*, *metB*, and *metC* genes of *C. glutamicum* and demonstrated the functionality of the transsulfuration pathway at genetic and biochemical levels. However, unlike the *metA* mutant of *C. glutamicum*, the *metB* and *metC* mutant strains showed methionine prototrophy, suggesting the presence of additional route(s) which may bypass the transsulfuration pathway (14, 19).

In this study, we provide evidence that the additional route is the direct sulfhydrylation pathway mediated by *metY*. The

* Corresponding author. Mailing address: Graduate School of Biotechnology, Korea University, Anam-dong, Sungbuk-ku, Seoul 136-701, Korea. Phone: 82-2-3290-3436. Fax: 82-2-923-9923. E-mail: hlee@korea.ac.kr.

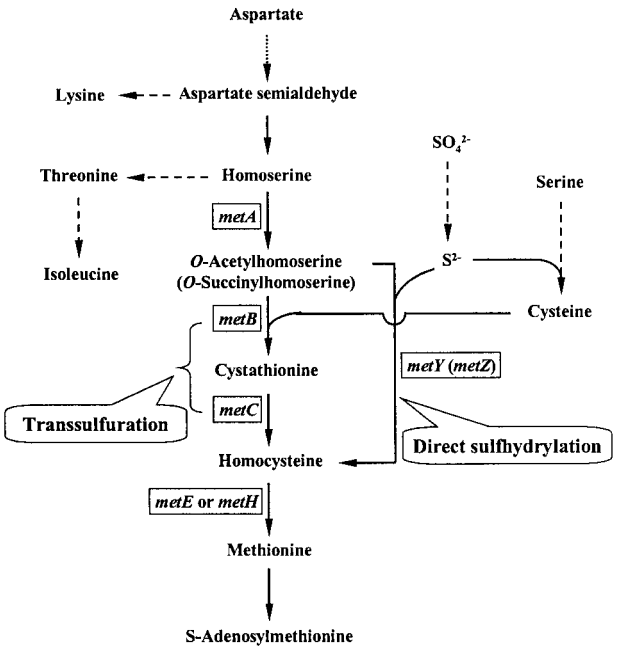

FIG. 1. Biosynthesis of methionine in eubacteria. In *E. coli*, the *metA* gene expresses homoserine succinyltransferase, which converts homoserine into *O*-succinylhomoserine. In *Bacillus subtilis, Brevibacterium flavum, C. glutamicum*, and *L. meyeri*, the *metA* gene expresses homoserine acetyltransferase which uses acetyl-CoA as the acyl donor to produce *O*-acetylhomoserine. Depending on the organism, formation of homocysteine from acylhomoserine can occur in two different routes. Transsulfuration is mediated by *metB* and *metC*, and direct sulfhydrylation is mediated by *metY* (or *metZ*). Unlike enteric bacteria, which preferentially use the transsulfuration pathway, *Brevibacterium flavum* (which is closely related to *C. glutamicum*) uses only the direct sulfhydrylation pathway to produce homocysteine.

molecular characteristics of the *metY* gene, encoding *O*-acetylhomoserine sulfhydrylase, are also investigated. Finally, based on biochemical and genetic evidence, we propose a methionine biosynthetic pathway of *C. glutamicum*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* and *C. glutamicum* cells were cultured at 37°C in Luria-Bertani (LB) medium (33) and at 30°C in MB (12), respectively. Minimal media for *E. coli* and *C. glutamicum* were M9 (33) and MCGC (45), respectively. Glucose was added to a 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 of biotin/liter. The sulfate concentration of the medium was adjusted with ammonium sulfate. Ampicillin and kanamycin were added to final concentrations of 50 and 25 mg/liter, respectively. All amino acids were added to a final concentration of 40 mg/liter.

DNA technology. Standard molecular cloning, transformation, and electrophoresis procedures were used (33). ExTaq DNA polymerase, restriction endonucleases, and modifying enzymes were purchased from Takara (Takara Shuzo Co., Tokyo, Japan) and used as described in the manufacturer's instructions. *E. coli* DH5αF′ and *C. glutamicum* AS019E12 were used as hosts for typical transformation. Transformation of *C. glutamicum* was performed by electroporation by using the methods of van der Rest et al. (44), and transformants were selected in MB containing kanamycin. Plasmid preparation for *C. glutamicum* was performed as previously described (49).

For nucleotide sequence analysis of *metY*, plasmids pSL72 and pSL73 were used as templates. The complete nucleotide sequence of *metY* was determined commercially at the Korea Research Center for Basic Sciences (Taejon, Korea) with universal and synthetic oligonucleotide primers. A sequence similarity search of nucleotide and amino acid sequences was performed at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) by using the basic local alignment search tool (BLAST) (1). Pairwise sequence alignments were performed at the website of ExPASy Proteomics Tools (http://www.expasy.ch/tools/) by using the CLUSTAL W alignment method (43).

Construction of plasmids. Plasmid pSL191 was constructed by inserting the blunt-ended 2.2-kb *Sph*I-*Sca*I fragment of pSL73 into the *Sma*I-digested pMT1. Plasmids pSL312 and pSL315 were constructed by the crossover PCR method as described previously (22). The primers used are listed in Table 1, and their annealing regions are shown in Fig. 6A. The primary PCR products, AB and CD, were amplified with 600 nM concentrations of the outer primers and 60 nM concentrations of the inner primers. These products were directly used as templates for the secondary PCR with 600 nM concentrations of the outer primers. Secondary PCR products were digested with *Bam*HI and ligated into the *Bam*HI-digested pK19mobsacB. The ligation mixture was used to transform *E. coli* DH5αF′, and the transformants were selected on LB plates containing 25 mg of kanamycin and 40 mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)/liter.

Site-specific gene disruption. Site-specific gene disruption was performed by using the nonreplicable integration vector pK19mobsacB, which enables marker-free deletion of the target gene (34). Plasmids pSL312 and pSL315 were introduced into *C. glutamicum* AS019E12 by electroporation (44). Integration of the introduced plasmid into the chromosome by a single crossover was monitored on LB plates containing 25 mg of kanamycin/liter. The single crossover was confirmed by the inability of the cells to grow on LB plates containing 10% sucrose. For the deletion of the target gene and the vector by another round of a crossover, the kanamycin-resistant (Km^r) and sucrose-sensitive cells were grown for 8 h in the LB medium and spread onto LB plates containing 10% sucrose. Cells growing on the plates were picked and tested for the deletion of the target gene by PCR.

Biochemical analysis. Cell extracts were prepared as described previously (15). *O*-Acetylhomoserine was synthesized by the method of Nagai and Flavin (27). The activities of cystathionine γ-synthase were determined by the method of Ravanel et al. (30), which measures the disappearance of cysteine by the ninhydrin reaction. *O*-Acetylhomoserine sulfhydrylase was assayed as homocysteine formation by using the nitroprusside reaction (10). Protein was measured by the method of Bradford (5), with bovine serum albumin as the standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (21).

GenBank accession number. The nucleotide sequence of *metY* was deposited in GenBank under accession number AF220150.

RESULTS

Characteristics of the *C. glutamicum metY* gene. We previously isolated and characterized the *metA* gene required for methionine biosynthesis in *C. glutamicum* (29). In order to find additional *met* genes, we extended our sequence analysis to the upstream and downstream region of the *metA* gene by using plasmids pSL72 and pSL73 (Fig. 2A) as the templates. In the immediate upstream of *metA,* we found an open reading frame (ORF) that consisted of 1,314 bp and was separated by 143 bp from the *metA* gene (Fig. 2B). The ATG start site was chosen based on the similarities of the amino acid sequences with other *O*-acetylhomoserine sulfhydrylases. Judging from the length of the ORF, no other upstream ATGs were expected to serve as the start site. A potential ribosome-binding site (36) of AGGA was located 8 bp upstream from the ATG (Fig. 2B). A sequence of TAG was identified as the stop codon for the ORF. The GC content of the ORF was 57%, which is typical of *C. glutamicum* genes. The codon preference was also very similar to that of previously reported corynebacterial genes and, interestingly, it also indicated that the ORF could encode a protein that is expressed at a high level (24).

The putative gene product consisted of 437 amino acids encoding a 46,751-Da protein with a predicted isoelectric point

TABLE 1. Bacterial strains, plasmids, and primers

Strain or plasmid	Relevant genotype, phenotype, or sequence[a]	Source or reference
E. coli		
DH5αF′	ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 deoR endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1*	Bethesda Research Laboratory
CGSC 2569	Δ(*gpt-proA*)*62 lacY1 tsx-29 glnV44 galK2 hisG4 xylA5 mtl-1 argE3 thi-1 metA28*	CGSC[b]
CGSC 4896	*argF58 relA1 spoT1 metB1*	CGSC
CGSC 6684	*ecfA4 metC186 spoT1 thi-1*	CGSC
C. lactofermentum ATCC 13869	Glutamic acid producer	ATCC[c]
B. flavum ATCC 14067	Glutamic acid producer	ATCC
C. glutamicum		
ATCC 13032	Wild type	ATCC
AS019	Spontaneous rifampin-resistant mutant of ATCC 13059	49
AS019E12	Restriction-deficient variant of AS019	11
HL921	*metY*-disrupted AS019E12	This study
HL938	*metB*-disrupted AS019E12	This study
HL939	*metY metB* double-disrupted AS019E12	This study
Plasmids		
pUC19	Cloning vector, Ap^r	48
pK19mobsacB	Integration vector, *mob sacB* Km^r	34
pMT1	*E. coli-C. glutamicum* shuttle vector, Ap^r Km^r	12
pSL72	pMT1 carrying *metY* and *metA*	29
pSL73	pMT1 carrying *metY* and *metA*	29
pSL75	pMT1 carrying 2.0-kb *Xho*I-*Sal*I fragment of pSL73, *metA*	29
pSL79	pMT1 carrying 3.0-kb *Sph*I-*Sal*I fragment of pSL73, *metY* and *metA*	29
pSL90	pMT1 carrying 2.0-kb *Xho*I-*Sca*I fragment of pSL72, *metY*	29
pSL123	pMT1 carrying *metB*	14
pSL191	pMT1 carrying 2.2-kb *Sph*I-*Sca*I fragment of pSL73, *metY*	This study
pSL312	pK19mobsacB carrying Δ*metY*	This study
pSL315	pK19mobsacB carrying Δ*metB*	This study
Oligonucleotides[d]		
ppYA	5′-CGC*GGATCC*ATGGGTGTTTCTGTATGC-3′	This study
ppYB	5′-CCCATCCACTAAACTTAAACAGGTAGCGATGGTGTTGTC-3′	This study
ppYC	5′-TGTTTAAGTTTAGTGGATGGGACCACCACCCATTCACAG-3′	This study
ppYD	5′-CGC*GGATCC*TGCAGCCGGATTCGTAGT-3′	This study
ppBA	5′-CGC*GGATCC*GACTGTTTCAGAAGTGAT-3′	This study
ppBB	5′-CCCATCCACTAAACTTAAACAGATGTGATCGCCCGGCTT-3′	This study
ppBC	5′-TGTTTAAGTTTAGTGGATGGGAATCACCCAGGCCACGAA-3′	This study
ppBD	5′-CGC*GGATCC*GACTACACCTTTGACAAT-3′	This study

[a] r superscript indicates resistance. Ap, ampicillin.
[b] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[c] ATCC, American Type Culture Collection, Rockville, Md.
[d] Sequences shown in italics indicate *Bam*HI restriction sites for cloning into the integration vector pK19mobsacB. Underlined sequences are the annealing region for the secondary PCR (22).

of 5.0. The translated amino acid sequence of the ORF was compared with the sequences in the protein database. Among the known proteins, the putative *O*-succinylhomoserine (thiol)-lyase of *Mycobacterium tuberculosis* (7) gave the highest score, with an amino acid identity of 58% (Fig. 3). *O*-Acetylhomoserine sulfhydrylases of *L. meyeri* and *Bacillus halodurans* (2, 40) showed 47% identity. Although the identical amino acids were fairly well distributed throughout the sequences, close analysis of the amino acid sequences revealed seven conserved motifs that may be involved in the catalytic activity of the enzyme. In addition, a binding motif for pyridoxal 5′-phosphate was identified (Fig. 3). We named the corynebacterial gene *metY*, based on its amino acid sequence similarities.

Although located in close proximity, the *metA* gene appeared to be expressed independently of the *metY* gene, since a putative rho-independent transcriptional stop signal was found at the downstream region of *metY* gene , as shown in Fig. 2. In addition, deleting the *metY* gene did not affect the *metA* expression and vice versa (Fig. 2 and Table 2).

Complementation of *E. coli metB* and *metA* mutants. To test for the possibility of complementation, we introduced the *C. glutamicum metY* gene into *E. coli* CGSC 4896, an *E. coli metB* mutant strain, and tested for the growth of the strain on a minimal medium. When the complementation tests were performed at 37°C, a clone carrying both *metY* and *metA* (*metY-metA*) could complement the *E. coli metB* mutant strain, although a clone carrying only the *metY* or *metA* gene could not (Fig. 2). However, as shown in Fig. 4A, the complemented

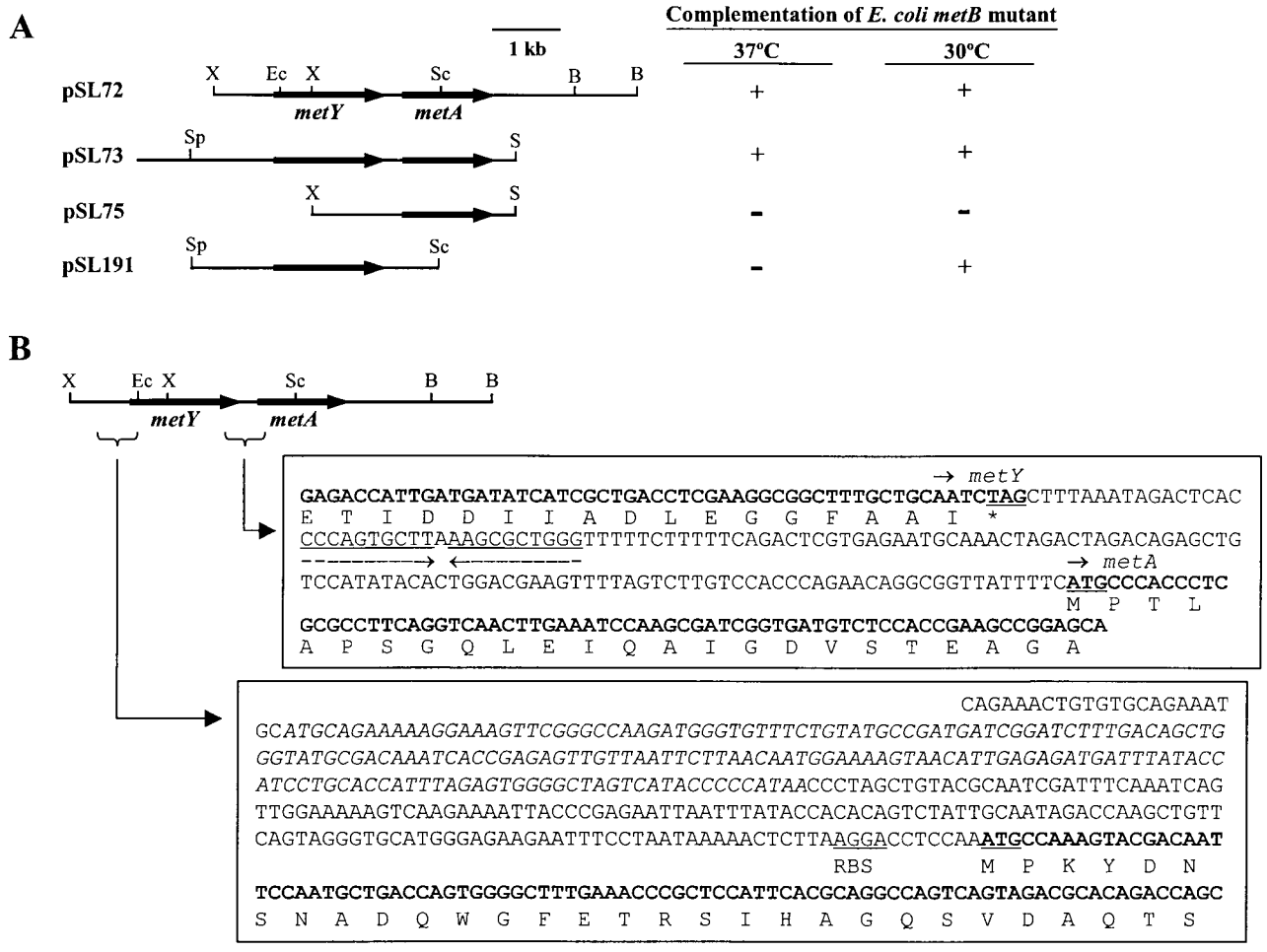

FIG. 2. Clones and subclones of *metY* (A) and nucleotide sequences of the *metY*-flanking regions (B). (A) *E. coli metB* mutant cells were transformed with the clones and tested for growth on the M9 minimal media. (B) Nucleotide sequences of the upstream and downstream region of *metY* gene. RBS, a putative ribosome-binding site. Sequences underlined with facing arrows indicate a putative rho-independent transcription stop signal. Sequences shown in italics indicate a putative leader sequence. Abbreviations: B, *Bam*HI; Ec, *Eco*O109I; S, *Sal*I; Sc, *Sca*I; Sp, *Sph*I; X, *Xho*I.

strain showed a long lag period of 60 h. The observed growth rate of the strain complemented with the clone carrying *metY-metA* was 0.184 h^{-1}, which is 50% slower than that of the strain complemented with the clone carrying *C. glutamicum metB* gene. However, as shown in Fig. 4B, the long lag disappeared soon when the complementation test was performed at 30°C, suggesting that the *metA* product of *C. glutamicum* may show temperature sensitivity, as found with those of *E. coli* (31) and *Bacillus polymyxa* (46). Unlike the complementation of the *E. coli metB* mutation with corynebacterial *metY* at 37°C, some degree of growth was observed at 30°C (Fig. 4B), which may also suggest temperature sensitivity of the *metY* product. For *E. coli* CGSC 2569, a *metA* mutant, a fragment carrying the *C. glutamicum metY-metA* complemented better than the one carrying only the *metA* gene, and it was the same at 37°C (Fig. 4C) or 30°C (data not shown). The observed growth rate at 37°C after complementation by the *metA* gene was 0.074 h^{-1}, only a 51% level compared to that achieved by the *metY-metA* genes. The enzyme activities of homoserine acetyltransferase, the product of the *metA* gene, were similar in these two strains. *E. coli* CGSC 2569 cells carrying *metY-metA* or *metA* showed specific activities of 694 and 768 nmol mg^{-1} min^{-1}, respectively, showing that the different growth pattern was not caused by the enzyme activity. Even though these data suggest that the methionine biosynthesis of *C. glutamicum* shows similarities in the overall pathways to that of *E. coli,* it also shows a clear difference presumably mediated by the corynebacterial *metY*. Based on these data, we concluded that (i) *O*-acetylhomoserine produced by the corynebacterial homoserine acetyltransferase (*metA*) is inefficiently utilized in *E. coli*; (ii) while the protein product of the *metY* gene utilize *O*-succinylhomoserine very poorly as the substrate, it can efficiently use *O*-acetylhomoserine produced from the corynebacterial *metA*; (iii) the *metY* gene of *C. glutamicum* encodes an enzyme that can replace the *E. coli metB* or *metC* gene product, but with different substrate specificity; and (iv) the *metY* gene may encode *O*-acetylhomoserine sulfhydrylase catalyzing the direct sulfhydrylation pathway which is not present in *E. coli*. Our

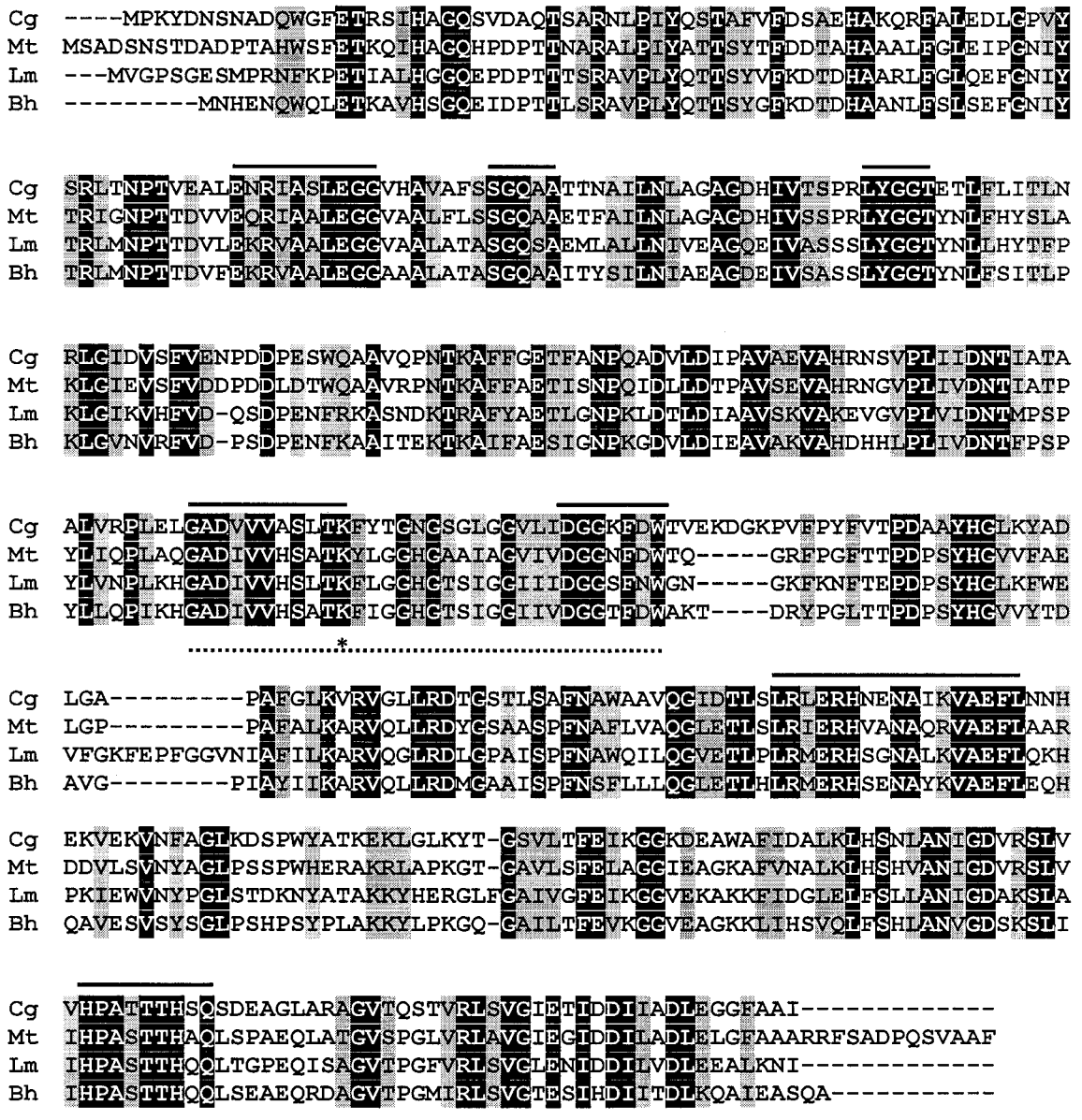

FIG. 3. Multiple sequence alignment of *O*-acetylhomoserine sulfhydrylases. Conserved and functionally similar amino acids are indicated by black and shaded boxes, respectively. Conserved motifs are indicated by solid bars. The binding motif for pyridoxal 5′-phosphate is shown with a dotted bar. An asterisk indicates the lysine residue which binds pyridoxal 5′-phosphate. Abbreviations: Cg, *O*-acetylhomoserine sulfhydrylase of *C. glutamicum* (AF220150); Mt, *O*-succinylhomoserine (thiol)-lyase of *M. tuberculosis* (AL021841); Lm, *O*-acetylhomoserine sulfhydrylase of *L. meyeri* (T44655); Bh, *O*-acetylhomoserine sulfhydrylase of *Bacillus halodurans* (AP001516).

effort to complement *E. coli metC* mutant with either *metY-metA* or *metY* was unsuccessful due to the leakiness of the *metC* mutation (37).

Expression of *O*-acetylhomoserine sulfhydrylase from *metY*. The ability of the *metY* clones to express *O*-acetylhomoserine sulfhydrylase was tested by enzymatic assays. Crude extracts were prepared from the various *C. glutamicum* AS019E12 cells harboring *metY* and/or *metA* clone(s) and assayed (Table 2). Introduction of the *metY* clone pSL73 or pSL191 into *C. glutamicum* AS019E12 increased *O*-acetylhomoserine sulfhydrylase activity ca. 30-fold. As evidenced by the complementation analysis, no *O*-acetylhomoserine sulfhydrylase activity was detected with *O*-succinylhomoserine as the substrate (data not shown). SDS-PAGE analysis of the crude extracts obtained from the *C. glutamicum* ASO19E12 cells harboring plasmid pSL73 or pSL191 revealed a putative *O*-acetylhomoserine sulfhydrylase band with an approximate M_r of 47,000 (Fig. 5). The data are in good agreement with the predicted molecular mass of 46,751 Da. The intensity of the protein band was roughly proportional to the activities observed in Table 2. The presence or absence of the protein band agreed with the enzymatic assay data. It is unique to observe an approximate 30-fold increase in the *O*-acetylhomoserine sulfhydrylase activity by the introduction of cloned *metY* gene into *C. glutamicum*, since

TABLE 2. Enzymatic activities of *O*-acetylhomoserine sulfhydrylase and cystathionine γ-synthase[a]

Strain	Plasmid	Phenotype	Sp act (nmol min^{-1} mg^{-1}) of:		Growth on minimal medium[b]
			OAHSH	CGS	
AS019E12					
	pMT1	Empty vector	14	17	+
	pSL73	*metY-metA* clone	426	ND	+
	pSL75	*metA* clone	17	ND	+
	pSL191	*metY* clone	415	ND	+
	pSL123	*metB* clone	ND[d]	113	+
HL921		Δ*metY*[c]	1.4	13	+
HL938		Δ*metB*[c]	10.1	4.0	+
HL939		Δ*metY* Δ*metB*[c]	1.4	3.8	−

[a] The enzymes were induced by growth of *C. glutamicum* cells to the stationary phase on MCGC minimal medium containing 1% glucose. Cells were harvested, disrupted, and assayed for activity as described in Materials and Methods. Enzyme reactions were carried out for 10 min at 30°C. The enzyme activities represent the mean of three independent experiments. OAHSH, *O*-acetylhomoserine sulfhydrolase; CGS, cystathione γ-synthase.
[b] MCGC minimal medium was used. Growth was monitored on agar plates.
[c] The mutants were generated in this study.
[d] ND, not determined.

it is common to observe only a 7- to 9-fold amplification for other genes cloned into the vector pMT1 (12). For example, introduction of the *metB* cloned pSL123 into *C. glutamicum* achieved a sevenfold increase in the cystathionine γ-synthase activity (Table 2). Introduction of the clones carrying *metA* and *metY*, such as pSL72 and pSL73, into the *C. glutamicum* AS019E12 cells resulted in the expression of an additional polypeptide with approximate M_r of 41,000 (Fig. 5). The protein band was not detected in the extracts of the cells carrying plasmid pSL191, a clone that does not carry *metA* (Fig. 2). The result indicates that the additional band may be homoserine acetyltransferases expressed from the *metA* gene. The level of the protein *O*-acetylhomoserine sulfhydrylase expression achieved by the *metY* gene was higher than that of the protein homoserine acetyltransferase by the *metA* gene, as evidenced by the strong intensity of the protein band (Fig. 5) and higher enzymatic activities of *O*-acetylhomoserine sulfhydrylase (46.1 nmol min^{-1} mg^{-1}) than those of homoserine acetyltransferase (32.7 nmol min^{-1} mg^{-1}). The data agree with the codon preference data (24), which indicated an efficient expression of the *metY* gene, and they also suggest a greater efficiency in the expression of the *metY* gene compared to that of the *metA* gene.

Evidence for the direct sulfhydrylation pathway in *C. glutamicum*. To address the role of *metY*, we constructed a *metY* mutant strain by using the cloned gene. The constructed mutant strain (HL921) carried an internally deleted *metY* gene (Fig. 6). As it was observed in *metB* (14) and *metC* (19) mutant strains of *C. glutamicum* HL921 also showed prototrophy for methionine (Fig. 6B), but the *O*-acetylhomoserine sulfhydrylase activity of the strain was negligible (Table 2). Because *C. glutamicum* HL938, a *metB* mutant strain, also showed methionine prototrophy (Fig. 6B), we constructed a *metB metY* double mutant strain by using the technique developed by Schäfer et al. (34) and tested for methionine requirement. Unlike the single mutant strains, as shown in Fig. 6B, the *metB metY* double mutant strain (HL939) was unable to grow on a minimal medium lacking supplemental methionine. Both the *O*-acetylhomoserine sulfhydrylase and the cystathionine γ-synthase activities of the double mutant strain were negligible (Table 2). Supplementation with homocysteine or methionine was enough to support the growth of the mutant strain (data not shown). Additionally, the growth characteristics of both

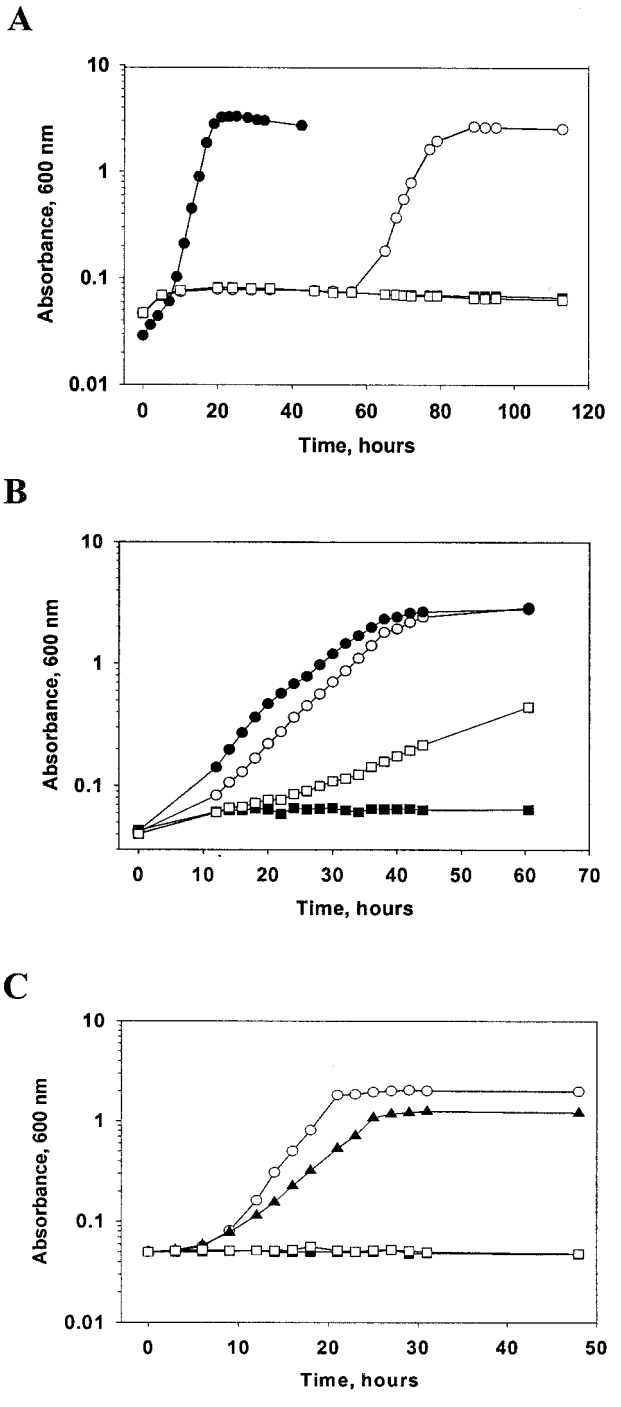

FIG. 4. Complementation of *E. coli metB* and *metA* mutants. Clones of *metY*, *metA*, *metY-metA*, or *metB* were transformed into *E. coli metB* (A and B) or *metA* (C) mutant strains, and the transformed strains were grown in M9 minimal medium lacking supplemental methionine at 37°C (A and C) or 30°C (B). Growth measurements were performed with two independent cultures. Symbols: ■, pMT1 (empty vector); □, pSL191 (*metY*); ●, pSL123 (*metB*); ○, pSL73 (*metY metA*); ▲, pSL75 (*metA*).

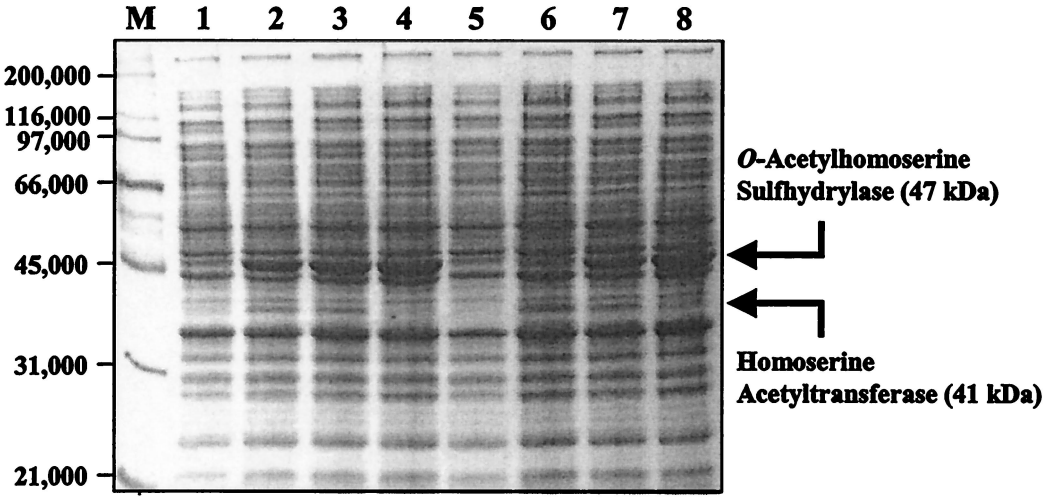

FIG. 5. Expression of *O*-acetylhomoserine sulfhydrylase and homoserine acetyltransferase from various *metY* and/or *metA* clones. Plasmids were introduced into the *C. glutamicum* AS019E12 cells, and crude extracts were prepared from the cells grown in the MCGC minimal media. Proteins were separated by SDS–12% PAGE. The protein bands corresponding to the *O*-acetylhomoserine sulfhydrylase (*metY*) and homoserine acetyltransferase (*metA*) are indicated. Lane M, molecular size markers; lane 1, AS019E12/pMT1 (empty vector); lane 2, AS019E12/pSL72 (*metY metA*); lane 3, AS019E12/pSL73 (*metY metA*); lane 4, AS019E12/pSL191 (*metY*); lane 5, HL921 (Δ*metY*); lane 6, AS019E12/pSL75 (*metA*); lane 7, AS019E12/pSL79 (*metY metA*); lane 8, AS019E12/pSL90 (*metY*).

metY and *metB* single mutants in MB and minimal medium were identical (data not shown). These data clearly show the functionality of the *metY* gene in *C. glutamicum* and also demonstrate, in addition to the transsulfuration mediated by *metB* gene, the presence of direct sulfhydrylation pathway, mediated by *metY*, for methionine biosynthesis in *C. glutamicum* (Fig. 7).

We also examined the presence of direct sulfhydrylation and transsulfuration pathways in bacteria related to *C. glutamicum*. Like *C. glutamicum* AS019E12, enzyme activities of *O*-acetylhomoserine sulfhydrylase (*metY*) and cystathionine γ-synthase (*metB*) were detected in several coryneform bacteria, such as *C. glutamicum* ATCC 13032, *C. lactofermentum* ATCC 13869, and *Brevibacterium flavum* ATCC 14067, and the level of activity was comparable to that of *C. glutamicum* AS019E12 (data not shown).

Regulation of *metY* expression. As the first step to investigate the role of direct sulfhydrylation pathway, we examined the effect of various amino acids on the expression of the *metY* gene. Among the aspartate family of amino acids tested, such as methionine, *S*-adenosylmethionine, threonine, lysine, isoleucine, and leucine, only methionine was effective to repress the expression of *metY*. The addition of 0.5 mM methionine to the MCGC minimal medium resulted in a 99% reduction in the *O*-acetylhomoserine sulfhydrylase activity (Table 3). The gene *metB* also appeared to be repressed by methionine, but the degree of repression was less than that by caused *metY* (Table 3). In agreement with the data, only the marginal activity of *O*-acetylhomoserine sulfhydrylase was also detected from the cells grown in a complex medium. The activity of cystathionine γ-synthase observed in the same cells was not changed significantly compared to that observed in the cells grown in a synthetic medium (Table 3). Assuming that sulfur is incorporated through the direct sulfhydrylation pathway, we tested the effect of sulfate concentration on the expression of *metY*. Although sulfate was added to the growth media up to 40 mM, no differences in growth pattern and *O*-acetylhomoserine sulfhydrylase activities were detected (data not shown). Similar results were obtained with cysteine.

DISCUSSION

In this report, we describe the presence of two parallel pathways, called transsulfuration and direct sulfhydrylation, for methionine biosynthesis in *C. glutamicum* (Fig. 7). The presence of the direct sulfhydrylation pathway in *C. glutamicum* was shown by (i) the complementation of the *E. coli metB* strain with *metY-metA*, (ii) the expression of *O*-acetylhomoserine sulfhydrylase activity from the *metY* gene, (iii) the requirement of chromosomal *metY metB* double disruption for methionine auxotrophy, and (iv) the amino acid sequence similarity of the *metY* gene product with other *O*-acetylhomoserine sulfhydrylases. In addition, previous reports on the *metB* and *metC* mutants of *C. glutamicum* which showed methionine prototrophy (14, 19) also support our findings. The presence of *O*-acetylhomoserine in the direct sulfhydrylation pathway was shown by the complementation of *E. coli metA* and *metB* mutants and by enzymatic assays. *C. glutamicum* shows uniqueness in methionine biosynthesis because it possesses functional transsulfuration and direct sulfhydrylation pathways and catalyzes each pathway with independent enzymes (Fig. 7). As evidenced in this work, direct sulfhydrylation and transsulfuration pathways also appear to be functional in related coryneform bacteria, such as *C. lactofermentum* and *Brevibacterium flavum. M. tuberculosis,* which is related to *C. glutamicum,* may also possess both pathways, since its *metB* mutant shows methionine prototrophy (39). Although *S. cerevisiae*, *Neurospora* spp., and green plants were reported to have enzyme activities of both pathways, unlike *C. glutamicum*, only one of the pathways might be physiologically meaningful and responsible for the methionine biosynthesis in the organism (8, 13, 18, 30, 42).

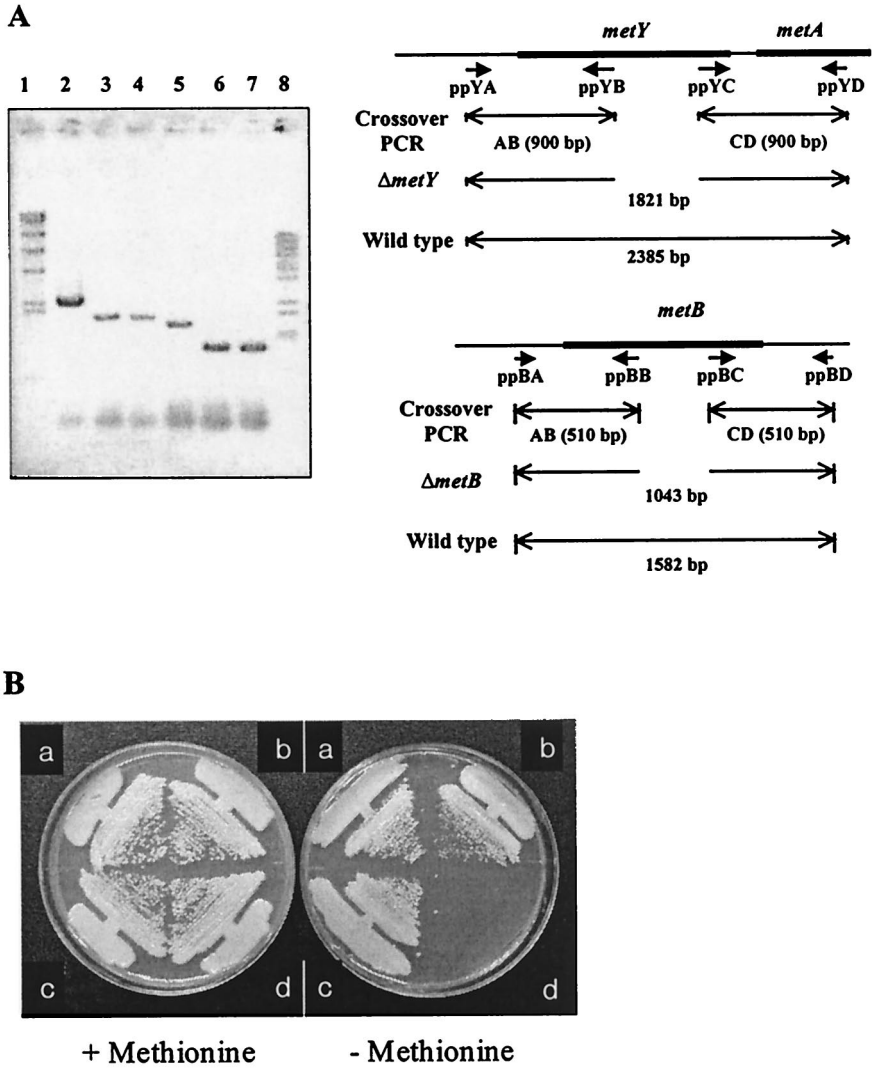

FIG. 6. Construction of *C. glutamicum* AS019E12 mutant in *metY* and *metB* genes (A) and growth properties of the mutants (B). (A) Chromosomal deletion of the gene was identified in agarose gels by PCR amplification. To test for the deletion, primers of ppYA and ppYD were used for *metY* (lanes 2, 3, and 4) and primers of ppBA and ppBD were used for *metB* (lanes 5, 6, and 7). The predicted lengths of the amplified fragment are shown. Lanes: 1, λ-*Hin*dIII size marker; 2 to 4, *C. glutamicum* AS019E12, HL921, and HL939, respectively; 5 to 7, *C. glutamicum* AS019E12, HL938, and HL939, respectively; 8, λ-*Bst*EII size marker. (B) Abilities of the constructed mutant strains to grow on MCGC minimal medium. The left plate contains supplemental methionine, whereas the right plate does not. a to d, *C. glutamicum* AS019E12, HL938, HL921, and HL939, respectively.

The methionine biosynthetic pathway of *C. glutamicum* also shows a clear distinction from that of *E. coli*, which is known to possess only the transsulfuration pathway (Fig. 7). In addition to the presence of the direct sulfhydrylation pathway in *C. glutamicum*, the enzymatic substrates for the *metA* and *metB* gene products of *C. glutamicum* are also different from those of *E. coli* (Fig. 7). Unlike *E. coli*, which favors succinylated substrates (8), *C. glutamicum* utilizes only acetylated substrates. Complementation of a *metB* mutation with a corynebacterial *metY metA* mutation, but very slow complementation with *metY* alone, shows that corynebacterial *O*-acetylhomoserine sulfhydrylase (*metY*) prefers *O*-acetylhomoserine to *O*-succinylhomoserine as the substrate. In addition, improved complementation of an *E. coli metA* mutation by a corynebacterial *metY metA* mutation shows that *O*-acetylhomoserine produced by corynebacterial *metA* can be more efficiently utilized by corynebacterial *O*-acetylhomoserine sulfhydrylase than *E. coli* cystathionine γ-synthase. Complementation of *E. coli metA* mutation by corynebacterial *metA* also confirms the previous findings that the *E. coli metB* gene product (cystathionine γ-synthase) also utilizes *O*-acetylhomoserine in place of its natural substrate, *O*-succinylhomoserine (8).

Interestingly, in many organisms, the *metY* and *metA* genes (or their homologs) appear to be located next to each other. This was true of *L. meyeri* (2, 4), *M. leprae*, *Thermotoga maritima*, *M. tuberculosis*, *Deinococcus radiodurans*, and *Thermus thermophilus.* Except for *L. meyeri*, the data were obtained by genome sequencing and therefore the physiological importance has not been addressed yet. Although the *metY* and *metA* genes of *C. glutamicum* were also located in close proximity to

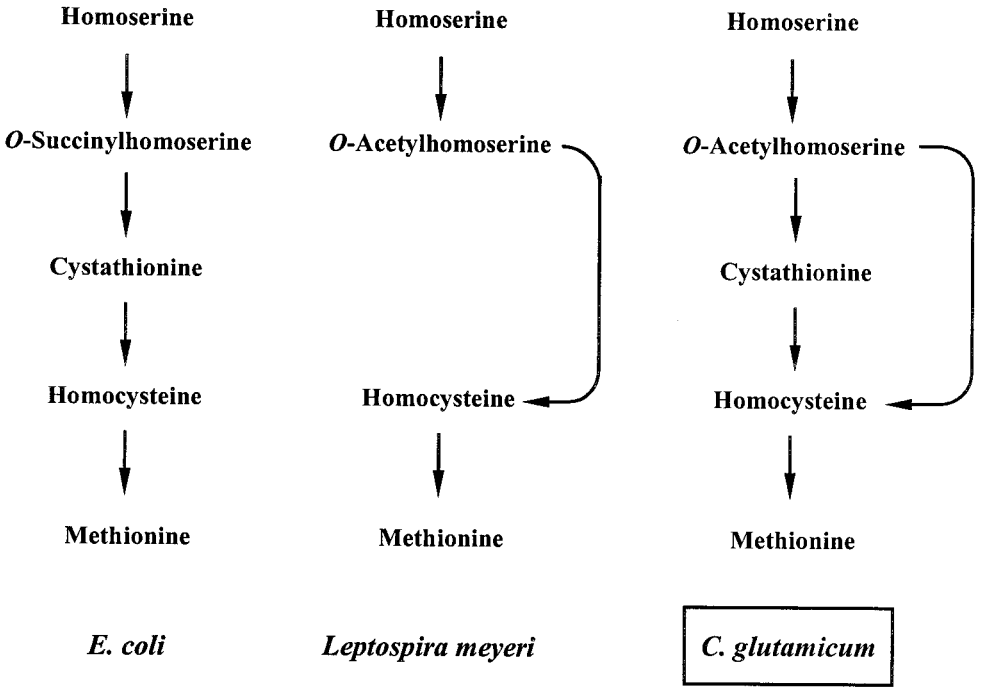

FIG. 7. Model for the methionine biosynthetic pathway in *C. glutamicum*. Unlike other pathways, both the transsulfuration and direct sulfhydrylation pathways appear to be functional in *C. glutamicum*, as shown in the present study.

each other, they appeared to be expressed independently. This hypothesis is supported by the deletion analysis performed in this work and the presence of a putative transcriptional terminator in the downstream of *metY*. If cells decide to utilize only the transsulfuration pathway, it will be unnecessary for them to express the *metY* gene. Therefore, the simultaneous expression of *metY* and *metA* genes will be energetically inefficient, since *metA* should always be expressed when methionine is absent but the expression of *metB* or *metY* is optional thereafter.

TABLE 3. Activities of *O*-acetylhomoserine sulfhydrylase and cystathionine γ-synthase in complex and minimal media containing methionine[a]

Culture condition	Concn (mM) of methionine added to the medium	Sp act (nmol min^{-1} mg^{-1}) of:	
		OAHSH	CGS
MCGC minimal medium	None	42	44
	0.1	7.8	23
	0.5	0.4	14
	5	0.4	10
	10	0.3	9
MB complex medium	None	1.1	19
	5	0.2	14
	10	0.2	14
	20	0.1	10

[a] The enzymes were induced by growing *C. glutamicum* AS019E12 cells to the stationary phase on the described medium containing the appropriate amount of methionine. When MCGC minimal medium was used, glucose was added to a final concentration of 1%. Cells were harvested, disrupted, and assayed for activity as described in Materials and Methods. Enzyme reactions were carried out for 10 min at 30°C. The enzyme activities represent the mean of three independent experiments. OAHSH, *O*-acetylhomoserine sulfhydrolase; CGS, cystathione *r*-synthase.

Thus, the independent expression of *metY* from *metA* is logical. This will be logical only if one of the *metB* or *metY* genes is expressed in a given physiological condition. In addition, as with the *O*-succinylhomoserine sulfhydrylase of *P. aeruginosa* (10), no methionine and cysteine residues were found in the coding region of *metY*. This suggests that amino acids or compounds containing sulfur may regulate *metY* expression. Although a putative leader sequence encoding a polypeptide composed of 60 amino acids was found in the upstream region of *metY*, the regulation mechanism of the gene expression by attenuation requires a further research.

Although we are uncertain about the importance of each pathway, our results suggest that the direct sulfhydrylation pathway is highly regulated. The evidence is for this is as follows: (i) the repressibility of *metY* gene by methionine, (ii) the unusually high level of *metY* expression in *C. glutamicum*, and (iii) the dramatic decrease of *metY* activity in complex media. These findings suggest that, compared to the transsulfuration pathway, the direct sulfhydrylation pathway is a low-affinity and energy-wasting route for methionine biosynthesis. Demonstration of our hypothesis will require further analysis with purified enzymes.

In conclusion, *C. glutamicum* possesses both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis (Fig. 7) and utilizes the pathways with almost equal efficiency, as evidenced by genetic and biochemical data. This situation is unique among prokaryotes and may provide evolutionary clues because methionine biosynthesis is known to show variations among organisms. Although we do not understand the role of each route, each pathway, analogous to the biosynthesis leading to lysine in *C. glutamicum* (35), which utilizes the succinylase or dehydrogenase pathway depending

on the availability of ammonium ion, may play a critical role under a given physiological condition.

ACKNOWLEDGMENTS

We thank Lothar Eggeling (Biotechnologie 1, Forschungszentrum Jülich, Jülich, Germany) for kindly providing us with pK19mobsacB.

This work was supported by grants from BASF Korea (to H.-S.L.) and Korea University (to H.-S.L.).

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Belfaiza, J., A. Martel, D. Margarita, and I. Saint Girons.** 1998. Direct sulfhydrylation for methionine biosynthesis in *Leptospira meyeri*. J. Bacteriol. **180:**250–255.
3. **Born, T. L., and J. S. Blanchard.** 1999. Enzyme-catalyzed acylation of homoserine: mechanistic characterization of the *Escherichia coli metA*-encoded homoserine transsuccinylase. Biochemistry **38:**14416–14423.
4. **Bourhy, P., A. Martel, D. Margarita, I. Saint Girons, and J. Belfaiza.** 1997. Homoserine *O*-acetyltransferase, involved in the *Leptospira meyeri* methionine biosynthetic pathway, is not feedback inhibited. J. Bacteriol. **179:**4396–4398.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Brzywczy, J., and A. Paszewski.** 1993. Role of *O*-acetylhomoserine sulfhydrylase in sulfur amino acid synthesis in various yeasts. Yeast **9:**1335–1342.
7. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, S. Oliver, J. Osborne, M. A. Quail, M. A. Rajandream, J. Rogers, S. Rutter, K. Seeger, S. Skelton, S. Squares, R. Squares, J. E. Sulston, K. Taylor, S. Whitehead, and B. G. Barrell.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
8. **Datko, A. H., J. Giovanelli, and S. H. Mudd.** 1974. Homocysteine biosynthesis in green plants. *O*-Phosphorylhomoserine as the physiological substrate for cystathionine γ-synthase. J. Biol. Chem. **249:**1139–1155.
9. **Eggeling, L., S. Morbach, and H. Sahm.** 1997. The fruits of molecular physiology: engineering the L-isoleucine biosynthesis pathway in *Corynebacterium glutamicum*. J. Biotechnol. **56:**167–182.
10. **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.
11. **Follettie, M. T., and A. J. Sinskey.** 1986. Recombinant DNA technology for *Corynebacterium glutamicum*. Food Technol. **40:**88–94.
12. **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.
13. **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.
14. **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. Cell **9:**300–308.
15. **Jetten, M. S. M., and A. J. Sinskey.** 1993. Characterization of phosphoenolpyruvate carboxykinase from *Corynebacterium glutamicum*. FEMS Microbiol. Lett. **111:**183–188.
16. **Jetten, M. S. M., and A. J. Sinskey.** 1995. Recent advances in the physiology and genetics of amino acid-producing bacteria. Crit. Rev. Biotechnol. **15:**73–103.
17. **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.
18. **Kerr, D. S., and M. Flavin.** 1970. The regulation of methionine synthesis and the nature of cystathionine γ-synthase in *Neurospora*. J. Biol. Chem. **245:**1842–1855.
19. **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. Cell **11:**220–225.
20. **Kinoshita, S.** 1985. Glutamic acid bacteria, p. 115–142. *In* A. L. Demain and N. A. Solomon (ed.), Biology of industrial microorganisms. The Benjamin/Cummings Publishing Company, London, England.
21. **Laemmli, U. K.** 1970. Cleavage of structure proteins during the assembly of head of bacteriophage T4. Nature **227:**680–685.
22. **Link, A. J., D. Phillips, and G. M. Church.** 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. J. Bacteriol. **179:**6228–6237.
23. **Malumbres, M., and J. F. Martín.** 1996. Molecular control mechanisms of lysine and threonine biosynthesis in amino acid-producing corynebacteria: redirecting carbon flow. FEMS Microbiol. Lett. **143:**103–114.
24. **Malumbres, M., J. A. Gil, and J. F. Martín.** 1993. Codon preference in corynebacteria. Gene **134:**15–24.
25. **Marzluf, G. A.** 1997. Molecular genetics of sulfur assimilation in filamentous fungi and yeast. Annu. Rev. Microbiol. **51:**73–96.
26. **Miyajima, R., and I. Shiio.** 1973. Regulation of aspartate family amino acid biosynthesis in *Brevibacterium flavum*. VII. Properties of homoserine *O*-transacetylase. J. Biochem. **73:**1061–1068.
27. **Nagai, S., and M. Flavin.** 1971. Synthesis of *O*-acetylhomoserine. Methods Enzymol. **17B:**423–424.
28. **Ozaki, H., and I. Shiio.** 1982. Methionine biosynthesis in *Brevibacterium flavum*: properties and essential role of *O*-acetylhomoserine sulfhydrylase. J. Biochem. **91:**1163–1171.
29. **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. Cell **8:**286–294.
30. **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.
31. **Ron, E. Z., and M. Shani.** 1971. Growth rate of *Escherichia coli* at elevated temperatures: reversible inhibition of homoserine trans-succinylase. J. Bacteriol. **107:**397–400.
32. **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.
33. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Schäfer, A., A. Tauch, W. Jäger, J. Kalinowski, G. Thierbach, and A. Pühler.** 1994. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. Gene **145:**69–73.
35. **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.
36. **Shine, J., and L. Dalgarno.** 1975. Determinant of cistron specificity in bacterial ribosomes. Naure **254:**34–38.
37. **Simon, M., and J.-S. Hong.** 1983. Direct homocysteine biosynthesis from *O*-succinylhomoserine in *Escherichia coli*: an alternate pathway that bypasses cystathionine. J. Bacteriol. **153:**558–561.
38. **Smith, D. A.** 1971. *S*-Amino acid metabolism and its regulation in *Escherichia coli* and *Salmonella typhimurium*. Adv. Genet. **16:**141–165.
39. **Smith, D. A., T. Parish, N. G. Stoker, and G. J. Bancroft.** 2001. Characterization of auxotrophic mutants of *Mycobacterium tuberculosis* and their potential as vaccine candidates. Infect. Immun. **69:**1142–1150.
40. **Takami, H., K. Nakasone, Y. Takaki, G. Maeno, R. Sasaki, N. Masui, F. Fuji, C. Hirama, Y. Nakamura, N. Ogasawara, S. Kuhara, and K. Horikoshi.** 2000. Complete genome sequence of the alkaliphilic bacterium *Bacillus halodurans* and genomic sequence comparison with *Bacillus subtilis*. Nucleic Acids Res. **28:**4317–4331.
41. **Taté, R., A. Riccio, E. Caputo, M. Iaccarino, and E. J. Patriarca.** 1999. The *Rhizobium etli metZ* gene is essential for methionine biosynthesis and nodulation of *Phaseolus vulgaris*. Mol. Plant-Microbe Interact. **12:**24–34.
42. **Thomas, D., and Y. Surdin-Kerjan.** 1997. Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **61:**503–532.
43. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
44. **van der Rest, M. E., C. Lange, and D. Molenaar.** 1999. A heat shock following electroporation induces highly efficient transformation of *Corynebacterium glutamicum* with xenogeneic plasmid DNA. Appl. Microbiol. Biotechnol. **52:**541–545.
45. **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.
46. **Wyman, A., E. Shelton, and H. Paulus.** 1975. Regulation of homoserine transacetylase in whole cells of *Bacillus polymyxa*. J. Biol. Chem. **250:**3904–3908.
47. **Yamagata, S.** 1989. Roles of *O*-acetyl-L-homoserine sulfhydrylases in microorganisms. Biochimie **71:**1125–1143.
48. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
49. **Yoshihama, M., K. Higashiro, E. A. Rao, M. Akedo, W. G. Shanabruch, M. T. Follettie, G. C. Walker, and A. J. Sinskey.** 1985. Cloning vector system for *Corynebacterium glutamicum*. J. Bacteriol. **162:**591–597.