J Ind Microbiol Biotechnol (2010) 37:1131–1136
DOI 10.1007/s10295-010-0760-3

ORIGINAL PAPER

Identification of a suppressor gene for the arginine-auxotrophic *argJ* mutation in *Corynebacterium glutamicum*

Gui-Hye Hwang · Jae-Yong Cho

Received: 6 March 2010 / Accepted: 25 May 2010 / Published online: 12 June 2010

Abstract We recently proposed a metabolic engineering strategy for L-ornithine production based on the hypothesis that an increased intracellular supply of *N*-acetylglutamate may further enhance L-ornithine production in a well-defined recombinant strain of *Corynebacterium glutamicum*. In this work, an *argJ*-deficient arginine auxotrophic mutant of *C. glutamicum* is suppressed by a different locus of *C. glutamicum* ATCC13032. Overexpression of the NCgl1469 open reading frame (ORF), exhibiting *N*-acetylglutamate synthase (NAGS) activity, was able to complement the *C. glutamicum* arginine-auxotrophic *argJ* strain and showed increased NAGS activity from 0.03 to 0.17 units mg^{-1} protein. Additionally, overexpression of the NCgl1469 ORF resulted in a 39% increase in excreted L-ornithine. These results indicate that the intracellular supply of *N*-acetylglutamate is a rate-limiting step during L-ornithine production in *C. glutamicum*.

Keywords *N*-acetylglutamate · *N*-acetylglutamate synthase · *Corynebacterium glutamicum* · L-ornithine production · Rate-limiting step

G.-H. Hwang · J.-Y. Cho
Department of Pharmaceutical Engineering,
College of Health Science, Sangji University,
660 Woosan-dong, Wonju-si,
Gangwon-do 220-702, Korea

J.-Y. Cho (✉)
labGENE Inc., Sangji University,
660 Woosan-dong, Wonju-si,
Gangwon-do 220-702, Korea
e-mail: jycho@sangji.ac.kr

Introduction

L-ornithine is an intermediate in the L-arginine biosynthetic pathway and has a wide range of potential applications in biotechnology. For example, it can be used as an important component in pharmaceuticals to stimulate the production and release of human growth hormone from the pituitary gland, to improve the immune response to bacteria and viruses, as well as to detoxify the body of ammonia deposits [1]. In *Corynebacterium glutamicum*, L-ornithine is biosynthesized from the precursor L-glutamate by the so-called cyclic pathway [2], in which the first step is transacetylation of glutamate catalyzed by the enzyme displaying *N*-acetylglutamate synthase (NAGS) activity encoded by an unknown gene [3], and L-ornithine is biosynthesized through the sequential activity of four enzymes encoded by the *argCJBD* genes. In this pathway, the acetyl group from acetylornithine is recycled with the generation of L-ornithine and *N*-acetylglutamate by ornithine acetyltransferase (OAT), the product of the *argJ* gene. In some microorganisms, the *argJ*-encoded product exhibits both NAGS and OAT activity [4, 5]. However, in *C. glutamicum*, the *argJ*-encoded product is considered as a monofunctional enzyme and lacks NAGS activity [3].

An organism, which regenerates *N*-acetylglutamate through L-ornithine synthesis, avoids the acetyl-CoA-consuming step for *N*-acetylglutamate synthesis catalyzed by NAGS. However, this enzyme activity may still be critical for an anaplerotic role in organisms using the cyclic pathway of L-ornithine synthesis in order to ensure a constant level of acetylated compounds during cell growth. In this study, we therefore attempt to functionally identify a gene, whose homologous expression provides the NAGS activity that supplies additional *N*-acetylglutamate for L-ornithine/L-arginine biosynthesis in *C. glutamicum*.

Materials and methods

Bacterial strains and growth conditions

Corynebacterium glutamicum ATCC13032 was employed for the construction of the mutant strains used in this study. For L-ornithine production experiments, a seed culture was made by inoculating cells into Recovery Glucose (RG) medium (80 g BHI, 20 g glucose, and 60 g sorbitol l^{-1} culture medium) and growing the cells overnight. Cells were harvested, washed, and resuspended in 10 ml of MMY medium [0.8 g KH_2PO_4, 10 g $(NH_4)_2SO_4$, 1 g $MgSO_4{\cdot}7H_2O$, 1.2 g Na_2HPO_4, 20 mg $MnSO_4{\cdot}H_2O$, 20 mg $FeSO_4{\cdot}7H_2O$, 10 mg $ZnSO_4{\cdot}7H_2O$, 10 g yeast extract, and 60 g glucose l^{-1} culture medium, pH 7.0] in a 100-ml baffled flask to an OD_{600} of 0.4–0.5, and then 0.2 g of sterile $CaCO_3$ per 10 ml of medium was aseptically added. Kanamycin was added to a final concentration of 50 μg ml^{-1} when appropriate. All cultures were grown at 30°C and 200 rpm on a rotary shaker, and samples were withdrawn at regular intervals for measurement of L-ornithine and biomass concentrations.

Plasmid and strain construction

The bacterial strains and plasmids constructed for this study are listed in Table 1. Oligonucleotide sequences used in this study are given in Table 1. Chromosomal DNA was isolated from *C. glutamicum* ATCC13032 and *Escherichia coli* W3110 as described previously [6] and used as a template in PCR using *Taq* or *Pfu* DNA polymerase to amplify DNA fragments of genes of interest.

To construct the *C. glutamicum argJ* gene-disrupted mutant strain, the internally deleted *argJ* gene was cloned into the pK18mobsacB integration vector [7], which enables marker-free deletion of the target gene, to generate the *argJ* gene disruption cassette. Site-specific gene disruption was performed by using this nonreplicable integration vector. Transformation of *C. glutamicum* was performed by electroporation using the methods of van der Rest et al. [8]. After integration of the plasmid, which is introduced into the chromosome by a single crossover, then plasmid excision from the chromosome via a second recombination in the presence of 10% sucrose results in either construction of the gene disrupted mutant or reconstitution of the wild-type genotype. Correct gene disruption of the chromosomal *argJ* gene in *C. glutamicum* was confirmed by a diagnostic PCR using gene-specific primers outside the regions of the targeted gene.

To construct strains overexpressing the genes involved in L-ornithine biosynthesis, the coding sequence of the corresponding gene and *trc* promoter region were first amplified by PCR using the *C. glutamicum* genomic DNA and the plasmid pTrc99A [9] as the templates, respectively, and the appropriate primer pairs (see Table 1). The PCR fragments were eventually cloned into the plasmid pEK0 [10] with the *rrnB* terminator PCR-amplified using pTrc99A as the template and the primer pairs TrrnBF-TrrnBR. The resulting plasmids, pEK-P_{trc}::0568, pEK-P_{trc}::1469, pEK-P_{trc}::2090, and pEK-P_{trc}::argJ, were transformed into *C. glutamicum* strains by electroporation, and the transformants were selected on RG plates containing kanamycin. Cells that grew on the selection plates were tested for the presence of plasmids by plasmid rescue and used to test the effects of gene expression on L-ornithine production.

The GenBank accession number of the DNA sequences, NCgl0568, NCgl1469, NCgl2090, and *argJ*, reported in this work is NC_003450.

Enzyme assays

Corynebacterium glutamicum cells were grown in MMY media, harvested by centrifugation, and washed in 100 mM Tris/HCl buffer (pH 7.5). Cells were disrupted using glass beads, and the resulting homogenate was centrifuged to obtain crude extract. All of these treatments were performed at 4°C. NAGS activity was measured in cell extracts by spectrophotometric determination of the formation of 5-thio-2-nitrobenzoate at 412 nm during the reaction between the free sulfhydryl group of CoASH, generated by the amino acid acetylating activity, and 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) [11]. One unit of NAGS activity will convert 1.0 nmole of glutamate and acetyl coenzyme A to acetylglutamate and coenzyme A in 1 min at pH 7.8 at 25°C.

Analytical methods

Cell growth in MMY broth was estimated by measuring OD_{600} using a spectrophotometer, and L-ornithine concentrations were determined by the colorimetric method with ninhydrin as described previously [12]. The values of L-ornithine production are reported as mg of L-ornithine per liter culture medium.

Results and discussion

Cloning of a putative gene with NAGS activity in *C. glutamicum*

In order to increase the *N*-acetylglutamate supply for L-ornithine biosynthesis in *C. glutamicum*, we first sought to identify a gene exhibiting NAGS activity that might ultimately limit the *N*-acetylglutamate. The whole genome

Table 1 Bacterial strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Description or sequence (5′–3′)[a]	Source, reference, or target[b]
C. glutamicum strains		
ATCC13032	*C. glutamicum* wild-type	This study
*argJ*Δ	*C. glutamicum* ATCC 13032, *argJ*Δ	This study
SJ8074	*C. glutamicum* ATCC 13032, *argF*Δ, *argR*Δ, *proB*Δ	[14]
Plasmids		
pEK0	*E. coli-C. glutamicum* shuttle vector, Km^R	[10]
pK18mobsacB	Mobilizable vector, *oriT sacB* Km^R	[7]
pTrc99A	Cloning vector for inducible expression of cloned inserts using the *trc* promoter in *E. coli*	[9]
pSJ996	pK18mobsacB derivative with 4,092-bp *Xba*I fragment of the *argCJBD* gene containing an internal in-frame deletion of the *Apa*I fragment of the *argJ* gene generated by PCR with primer pairs argCF-argJR5 and argJF3-argDR from the *C. glutamicum* ATCC13032 genomic DNA	This study
pEK-P_{trc}::0568	pEK0 with a 714-bp *Nde*I-*Hinc*II fragment of the NCgl0568 ORF encompassed by a 1,536-bp *Kpn*I-*Nde*I fragment of the *trc* promoter and a 407-bp *Sma*I-*Eco*RI fragment of the T_{rrnB} terminator	This study
pEK-P_{trc}::1469	pEK0 with a 741-bp *Nde*I-*Xba*I fragment of the NCgl1469 ORF encompassed by a 1,536-bp *Not*I-*Nde*I fragment of the *trc* promoter and a 407-bp *Sma*I-*Eco*RI fragment of the T_{rrnB} terminator	This study
pEK-P_{trc}::2090	pEK0 with a 761-bp *Nde*I-*Xba*I fragment of the NCgl2090 ORF encompassed by a 1,536-bp *Not*I-*Nde*I fragment of the *trc* promoter and a 407-bp *Sma*I-*Eco*RI fragment of the T_{rrnB} terminator	This study
pEK-P_{trc}::argJ	pEK0 with a 1,222-bp *Nco*I-*Xba*I fragment of the *C. glutamicum argJ* gene encompassed by a 1,578-bp *Sal*I-*Nco*I fragment of the *trc* promoter and a 407-bp *Sma*I-*Eco*RI fragment of the T_{rrnB} terminator	This study
Primers		
argCF	ctagtctagaTCCAGTTCAGGAAGCACC (*Xba*I)	*argC* (1845523–1845540)
argJR5	nnngggcccGTGTTTGCTGGTTAGGGC (*Apa*I)	*argC* (1844224–1844242)
argJF3	nnngggcccATGAACTCAACGATGCGG (*Apa*I)	*argJ* (1843454–1843471)
argDR	ctagtctagaCGAGCAAGTCGATGTAGAC (*Xba*I)	*argD* (1841900–1841918)
trcF	ctagtctagaTCATCACCGAAACGCGCGA (*Xba*I)	pTrc99A
trcR	ggaattccatatgTCTGTTTCCTGTGTGAAATTG (*Nde*I)	pTrc99A
0568F	ggaattccatatgAGTGAACAATTCGAGC (*Nde*I)	NCgl0568 (606392–606410)
0568R	ctagtctagaCAACGCTTCACGCATCAC (*Xba*I)	NCgl0568 (607088–607105)
1469F	ggaattccatatgAGTCCCACCGTTTTGCC (*Nde*I)	NCgl1469 (1610236–1610255)
1469R	ctagtctagaGCAGGAATCACATCTGACC (*Xba*I)	NCgl1469 (1610958–1610976)
2090F	catgcatgccatggGGAGCGTGTCACCGATC (*Nco*I)	NCgl2090 (2297870–2297997)
2090R	ctagtctagaACGAGATCCGCAAGGTGG (*Xba*I)	NCgl2090 (2298613–2298631)
argJF	catgcatgccatggCAGAAAAAGGCATTACCGC (*Nco*I)	*argJ* (1844170–1844191)
argJR	ctagtctagaCTAGAGTCATTCATGCCTGTGCC (*Xba*I)	*argJ* (1842978–1842989)
TrrnBF	cccccgggGCTGTTTTGGCGGATGAGAGAAG (*Sma*I)	pTrc99A
TrrnBR	cggaattcAAAAGGCCATCCGTCAGGATGGCC (*Eco*RI)	pTrc99A

[a] Underlined sequences indicate sites for restriction enzymes as shown in parentheses. Upper case letters refer to the sequences of bacterial genes. The *Nco*I restriction site was used for the trcR primer when needed. Other restriction sites including *Kpn*I, *Not*I, and *Sal*I are also available for the trcF primer

[b] Numerical position on *C. glutamicum* ATCC13032 (GenBank accession number NC_003450) is shown in parentheses

database of *C. glutamicum* was searched using BLASTP and based on homology to known NAGS; three putative NAGS genes, NCgl2090, NCgl1469, and NCgl0568, were revealed whose deduced amino acid sequences showed 34, 25, and 35% identities, respectively, to *Mycobacterium tuberculosis* NAGS (*Rv2747*), a member of the GCN5-related *N*-acetyltransferase superfamily. These putative NAGS genes, NCgl2090, NCgl1469, and NCgl0568, consisted of predicted coding sequences of 564, 612, and 510 bp, respectively. The open reading frames (ORFs)

were PCR-amplified from the *C. glutamicum* ATCC13032 genome using primers corresponding to their respective 5′- and 3′-ends. These PCR-amplified ORFs were cloned to form a fusion with the inducible *trc* promoter in plasmid pEK0 as described in Materials and Methods, generating the plasmids designated pEK-P_{trc}::2090, pEK-P_{trc}::1469, and pEK-P_{trc}::0568 for further analysis.

Identification of the homologous *C. glutamicum* gene for NAGS

To identify the gene(s) exhibiting NAGS activity, we tested whether the putative NAGS genes retained the ability to complement the arginine-auxotrophic mutant that could not supply *N*-acetylglutamate for L-ornithine/L-arginine biosynthesis. For this purpose, an arginine-auxotrophic mutant of *C. glutamicum* ATCC13032 was constructed by disrupting the *argJ* gene encoding OAT as described in the Materials and methods. All three plasmids were tested for their efficiency to complement the arginine deficiency of the mutant strain, using spot tests of serial dilutions in the presence of IPTG (Fig. 1). Under inducing conditions, pEK-P_{trc}::1469 and pEK-P_{trc}::argJ allowed growth of the arginine-deficient mutant in the absence of arginine, while pEK-P_{trc}::2090, pEK-P_{trc}::0568, and the empty vector pEK0 were unable to complement the arginine deficiency of the mutant. These results suggest that the overexpression of the NCgl1469 ORF plays an important role in the supply of *N*-acetylglutamate for L-ornithine/L-arginine biosynthesis.

Next, NAGS activity was determined in the *argJ* gene-disrupted mutant carrying the plasmid pEK-P_{trc}::1469. As shown in Table 2, the residual NAGS activity was detected in the *C. glutamicum* ATCC13032 background as described previously [3]. However, the *argJ* gene-disrupted mutant carrying pEK-P_{trc}::1469 showed a relatively high NAGS activity, which can be ascribed to overexpression of the plasmid-borne NCgl1469 ORF. On the other hand, no additional NAGS activity was detected for pEK-P_{trc}::argJ, which is expected if OAT acts as a monofunctional enzyme and lacks NAGS activity. This suggests that the

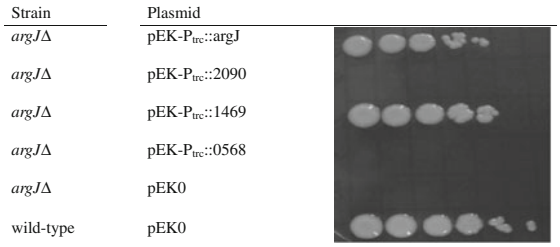

Fig. 1 Spot growth test of the *C. glutamicum argJ* gene-disrupted mutant carrying various plasmids as indicated. In each *row*, from *left* to *right*, 1 µl of tenfold serial dilutions of a cell suspension were spotted under inducing conditions with 10 mM IPTG

Table 2 NAGS specific activities of the *C. glutamicum argJ*Δ strain carrying different plasmids

C. glutamicum strain[a]	Plasmid	NAGS specific activity (units mg^{-1} protein)[b]
*argJ*Δ	pEK0	0.03
	pEK-P_{trc}::1469	0.17
	pEK-P_{trc}::argJ	0.05

[a] The plasmid-containing cells were grown in MMY medium with kanamycin in the presence of 1 mM arginine and 10 mM IPTG for 12 h. Cells carrying different plasmids showed almost the same growth rate

[b] The values are means of triplicate cultures, and the standard deviations were, in all cases, below 10%

C. glutamicum NCgl1469 ORF encodes a protein displaying NAGS activity, but that the endogenous NCgl1469 ORF is insufficient to express enzymatic activity. This abnormality likely contributes to the failure of *argJ* gene-disrupted mutant to grow in the absence of arginine. We also examined whether NAGS activity detected in the *argJ* gene-disrupted mutant carrying pE K-P_{trc}::1469 was affected in the presence of L-ornithine or L-arginine. Inhibition of NAGS activity by L-ornithine or L-arginine was tested by adding L-ornithine or L-arginine to the reaction mixture of the enzyme assay as described in Materials and methods. Although 50% inhibition of NAGS activity was observed in the presence of 0.2 mM L-ornithine, no inhibition could be observed in the presence of L-arginine (Fig. 2).

Effect of NCgl1469 ORF expression on the L-ornithine production in an L-ornithine-producing strain

In subsequent experiments, plasmid pEK-P_{trc}::1469 was transformed into the mutant strain SJ8074, which is

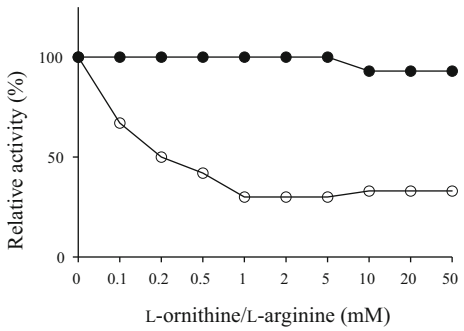

Fig. 2 Inhibition of NAGS activity of the *C. glutamicum argJ* gene-disrupted mutant carrying pEK-P_{trc}::1469 by L-ornithine or L-arginine. Symbols: *filled circle*, NAGS activity of the *C. glutamicum argJ* gene-disrupted mutant carrying pEK-P_{trc}::1469 in the presence of L-arginine; *open circle*, NAGS activity of the *C. glutamicum argJ* gene-disrupted mutant carrying pEK-P_{trc}::1469 in the presence of L-ornithin. The values are means of triplicate assays, and the standard deviations were, in all cases, below 10%

Table 3 L-Ornithine production in the *C. glutamicum* SJ8074 recombinant strain

C. glutamicum strain[a]	Plasmid	L-Ornithine production (mg l^{-1})[b]
SJ8074	pEK0	230
	pEK-P_{trc}::1469	320
	pEK-P_{trc}::argJ	224

[a] The plasmid-containing cells were grown in MMY medium with kanamycin in the presence of 1 mM arginine, 1 mM proline and 10 mM IPTG for 20 h. Cells carrying different plasmids showed almost the same growth rate

[b] The values are means of triplicate cultures, and the standard deviations were, in all cases, below 5%

deficient in citrulline and proline biosynthesis and blocked in the feedback repression by the arginine repressor ArgR, to test its relevance for L-ornithine production in the resulting recombinant strain. As shown in Table 3, overexpression of the NCgl1469 ORF resulted in an approximately 39% increase in L-ornithine production. However, overexpression of the *argJ* gene alone did not mimic the effect observed with the NCgl1469 ORF. These results suggest that L-ornithine production in strain SJ8074 is limited by an insufficient supply of *N*-acetylglutamate for L-ornithine biosynthesis. This limitation of *N*-acetylglutamate availability seems to be due to the operation of feedback inhibition by L-ornithine of the corynebacterial OAT as previously reported by Sakanyan et al. [3].

Based on the results of the homologous gene expression of the NCgl1469 ORF, we examined whether the NCgl1469 ORF encodes a protein that can be functionally redundant with NAGS. Complementation of the arginine-auxotrophic *argJ* mutation by the NCgl1469 ORF depends on the overexpression of the homologous gene, since the arginine-auxotrophic *argJ* mutant can be constructed from a cell that contains a single copy of the wild-type NCgl1469 ORF. These results reflect the consistent notion that many proteins retain the ability to catalyze identical biochemical reactions on numerous substrates in vivo [13].

The approach presented here allowed for the identification of a multicopy suppressor gene that can relieve a bottleneck in the downstream steps of the L-ornithine biosynthetic pathway. However, this approach resulted in only a 39% increase in the amount of excreted L-ornithine; thus, there is room for further improvement in L-ornithine production. Although overexpression of heterologous gene(s), such as the *argA* gene from *E. coli* mutants with feedback-resistant NAGS against L-arginine, for *N*-acetylglutamate synthesis is an option to enhance L-ornithine biosynthesis in *C. glutamicum*, there could potentially be problematic aspects to this approach since heterologous gene expression depends on components of the system of origin of the foreign genes. Enzyme assays using crude extract from the strain SJ8074 carrying plasmid pEK-P_{trc}::1469 revealed that the overexpressed NAGS activity was inhibited by relatively low concentrations of L-ornithine (Fig. 2), suggesting that the acetyltransferase reactions could still be limiting in this strain. Taken together, these results suggest that the effects of enzyme induction may still be dampened by the feedback inhibition of NAGS by L-ornithine in vivo, and a higher rate of carbon flow through the L-ornithine biosynthetic pathway will only be possible when the NAGS is relieved of feedback inhibition. An exploitation of relevant NAGS mutations that have feedback-resistant activity will address this problem, and we expect to identify unknown mutations that could have a prominent effect on L-ornithine production in the future.

Acknowledgments This research was supported by the Advanced R&D Supporting Business between Industry and University funded by the Small and Medium Business Administration, Republic of Korea, and in part by the Sangji University Research Fund 2009. The *lab*GENE Inc. was supported by a grant from the Gangwon Technopark.

References

1. Salvatore F, Cimino F, Maria C, Cittadini D (1964) Mechanism of the protection by L-ornithine-L-aspartate mixture and by L-arginine in ammonia intoxication. Arch Biochem Biophys 107:499–503
2. Cunin R, Glansdorff N, Pierard A, Stalon V (1986) Biosynthesis and metabolism of arginine in bacteria. Microbiol Rev 50:314–352
3. Sakanyan V, Petrosyan P, Lecocq M, Boyen A, Legrain C, Demarez M, Hallet J-N, Glansdorff N (1996) Genes and enzymes of the acetyl cycle of arginine biosynthesis in *Corynebacterium glutamicum*: enzyme evolution in the early steps of the arginine pathway. Microbiology 142:99–108
4. Sakanyan V, Kochikyan A, Mett I, Legrain C, Charlier D, Pierard A, Glansdorff N (1992) A re-examination of the pathway for ornithine biosynthesis in a thermophilic and two mesophilic *Bacillus* species. J Gen Microbiol 138:125–130
5. Sakanyan V, Charlier D, Legrain C, Kochikyan A, Mett I, Pierard A, Glansdorff N (1993) Primary structure, partial purification and regulation of key enzymes of the acetyl cycle of arginine biosynthesis in *Bacillus stearothermophilus*: dual function of ornithine acetyltransferase. J Gen Microbiol 139:393–402
6. Eikmanns BJ, Thum-Schmitz N, Eggeling L, Lüdtke K-U, Sahm H (1994) Nucleotide sequence, expression and transcriptional analysis of the *Corynebacterium glutamicum gltA* gene encoding citrate synthase. Microbiology 140:1817–1828
7. Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined selections in the chromosome of *Corynebacterium glutamicum*. Gene 145:69–73
8. van der Rest ME, Lange C, Molenaar D (1999) A heat shock following electroporation induces highly efficient transformation of *Corynebacterium glutamicum* with xenogeneic plasmid DNA. Appl Microbiol Biotechnol 52:541–545
9. Amann E, Ochs B, Abel KJ (1988) Tightly regulated *tac* promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene 69:301–315

10. Eikmanns BJ, Kleinertz E, Liebl W, Sahm H (1991) A family of *Corynebacterium glutamicum*/*Escherichia coli* shuttle vectors for cloning, controlled gene expression, and promoter probing. Gene 102:93–98
11. Errey JC, Blanchard JS (2005) Functional characterization of a novel ArgA from Mycobacterium tuberculosis. J Bacteriol 187:3039–3044
12. Chinard FP (1952) Photometric estimation of proline and ornithine. J Biol Chem 199:91–95
13. Patrick WM, Quandt EM, Swartzlander DB, Matsumura I (2007) Multicopy suppression underpins metabolic evolvability. Mol Biol Evol 24:2716–2722
14. Hwang J-H, Hwang G-H, Cho J-Y (2008) Effect of increased glutamate availability on L-ornithine production in *Corynebacterium glutamicum*. J Microbiol Biotechnol 18:704–710