Journal of Microbiology (2014) Vol. 52, No. 4, pp. 278–283
DOI 10.1007/s12275-014-3461-1

Analysis of *cepA* Encoding an Efflux Pump-like Protein in *Corynebacterium glutamicum*

Soo-Yeon Sim[1], Eun-Ji Hong[1], Younhee Kim[2], and Heung-Shick Lee[1]*

[1]*Department of Biotechnology and Bioinformatics, Korea University, Sejong 339-700, Republic of Korea*
[2]*Department of Oriental Medicine, Semyung University, Chungbuk 390-230, Republic of Korea*

(Received Sep 2, 2013 / Revised Nov 4, 2013 / Accepted Nov 6, 2013)

A gene encoding a homolog of purine efflux proteins of *Escherichia coli* and *Bacillus subtilis* was identified in the genome of *Corynebacterium glutamicum* and designated as *cepA*. The gene encoded a putative protein product, containing 12 transmembrane helixes, which is a typical feature of integral membrane transport proteins. To elucidate the function of the gene, we constructed a *cepA* deletion mutant (Δ*cepA*) and a *cepA*-overexpressing strain and analyzed their physiological characteristics. The *cepA* gene could be deleted with no critical effect on cell growth. However, the cell yield of a Δ*cepA* strain was decreased by 10% as compared to that of a strain carrying a *cepA*-overexpression plasmid (P_{180}-*cepA*). Further analysis identified increased resistance of the P_{180}-*cepA* strain to the purine analogues 6-mercaptopurine and 6-mercaptoguanine, but not to 2-aminopurine and purine nucleoside analogues. Moreover, this strain showed increased resistance to the antibiotics nalidixic acid and ampicillin. Collectively, these data suggest that *cepA* is a novel multidrug resistance gene and probably functions in the efflux of toxic substances from the inside of cells to the environment, thus allowing cells to reach a higher cell yield.

Keywords: *Corynebacterium glutamicum*, *cepA*, efflux, purine

Introduction

Corynebacterium glutamicum is a Gram-positive bacterium, and has been widely used for the fermentative production of amino acids and nucleotides (Leuchtenberger *et al*., 2005). While amino acids are typically used as flavor enhancers and feed supplements, purine nucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) are used as flavor enhancers. Due to the industrial importance of this organism, its genetic and biochemical features have been extensively characterized. Moreover, the recent availability of the genome sequence data has made it possible to characterize the genetic background of the organism in a new dimension.

Bacteria can express numerous transport proteins. The *C. glutamicum* genome contains over 200 genes that are assumed to be involved in the transport of a variety of molecules (Ikeda and Nakagawa, 2003; Kalinowski *et al*., 2003; Yukawa *et al*., 2007). Many of the transporters involved in amino acid efflux have been studied in detail (Eggeling and Sahm, 2003; Marin and Krämer, 2007). It is well known that overexpression of genes that encode these amino acid exporters improves the performance of the respective amino acid-producing *C. glutamicum* strains. In contrast to the studies on amino acid export, no information is available on *C. glutamicum* nucleosides exporters. Nucleosides are used as the raw material for synthesis of nucleotides. In *Bacillus subtilis*, the *pbuE* (*ydhL*) gene is known to encode the purine base and purine nucleosides efflux pump (Johansen *et al*., 2003; Zakataeva *et al*., 2007). It plays a role in maintaining an appropriate intracellular level of purine bases by ensuring an optimal balance between purine base utilization and purine biosynthesis. This gene also protects cells against toxic purine base analogues (Nygaard and Saxild, 2005), and helps stabilize the cellular energy charge (Zakataeva *et al*., 2007). In *Escherichia coli*, the *yicM* (*nepI*) gene is involved in the efflux of purine ribonucleosides such as inosine, but not of the free bases such as guanine and hypoxanthine (Gronskiy *et al*., 2005). Gronskiy *et al*. (2005) postulated that YicM mediates the export of purine ribonucleotides (formed as an rRNA degradation product) in the stationary phase. Recently, Sheremet *et al*. (2011) were able to enhance the production of inosine by introducing the *E. coli yicM* gene into a nucleoside-producing *Bacillus* strain.

Based on the above knowledge, in this study, we attempted to isolate the *C. glutamicum* genes involved in purine base and nucleoside export by employing bioinformatics tools. Subsequently, we identified the *cepA* gene and employed genetic approaches to understand its physiological role. Based on the results obtained, we propose a role for *cepA* and its potential use in *C. glutamicum* manipulation.

Materials and Methods

Bacterial strains, plasmids, and growth conditions

C. glutamicum AS019E12 (Follettie *et al*., 1993) was used to construct HL1264, which carries a ΔNCgl2903 (i.e. Δ*cepA*) mutation. The *C. glutamicum* HL1263 strain carries the NCgl2903-overexpressing plasmid pSL461 (i.e. P_{180}-*cepA*). *C. glutamicum* cells were routinely cultured at 30°C in MB

*For correspondence. E-mail: hlee@korea.ac.kr; Tel.: +82-44-860-1415; Fax: +82-44-864-2665

Table 1. ***C. glutamicum*** **proteins showing similarity to NepI and YdhL**

Proteins (Strains)	Gene ID (Protein ID)	Amino acids	Predicted function	Identity	Similarity
NepI (*E. coli*)	NCgl2903 (NP_602201)	392	Arabinose efflux permease	25%	45%
	NCgl0640 (NP_599902)	424	Sugar efflux permease	24%	40%
	NCgl2348 (NP_601631)	373	Permease of the MFS[a]	24%	40%
YdhL (*B. subtilis*)	NCgl0640 (NP_599902)	424	Sugar efflux permease	25%	43%
	NCgl2903 (NP_602201)	392	Arabinose efflux permease	24%	42%
	NCgl2592 (NP_601881)	481	Permease of the MFS	25%	36%

[a] MFS, major facilitator superfamily

(Follettie *et al.*, 1993) medium. The MCGC minimal medium for *C. glutamicum* culture was prepared as described previously (von der Osten *et al.*, 1989). *E. coli* cells were cultured at 37°C in LB (Sambrook and Russell, 2001). Carbon sources were added to the minimal medium at a final concentration of 1%. Antibiotics were added at the following concentrations (μg/ml): 50 ampicillin, 34 chloramphenicol, and 30 kanamycin.

DNA technology and strain construction

Routine DNA analysis involving *C. glutamicum* cells was performed as described previously (Park *et al.*, 2012). The *C. glutamicum* Δ*cepA* mutant strain was constructed according to the method described by Schäfer *et al.* (1994), as follows: A DNA fragment from the *C. glutamicum* genome was produced by crossover PCR utilizing the primers F1: 5′-TGAATTCTTCACAGCGGGC-3′, R1: 5′-CCCATCCA CTAAACTTAAACAGACATACGCGCTGCCATTG-3′, F2: 5′-TGTTTAAGTTTAGTGGATGGGCATACGCGCTC TCCCAA-3′, and R2: 5′-TCGAATTCGGGTGCGCGCAA CCTCT-3′. The amplified fragment was then digested with *Eco*RI and introduced into *Eco*RI-digested pK19mobsacB (Schäfer *et al.*, 1994). Subsequent steps were conducted as described by Schäfer *et al.* (1994) and Hwang *et al.* (2002), and the chromosomal deletion of *cepA* in *C. glutamicum* HL1264 was confirmed via PCR. The pSL461 plasmid, which overexpresses *cepA*, was constructed via the amplification of the *cepA* gene using the primers 5′-ACCTGCAGCCCA TTAAACAGCCCGATTC-3′ and 5′-ACCTGCAGGTGAC TTTTGGGTGGTTTTGG-3′, followed by ligation of the amplified DNA into the *Pst*I site of pSL360 (Park *et al.*, 2004).

Physiological and biochemical analysis

The sensitivity of the *C. glutamicum* cells to analogues was assessed on MB plates (Lee *et al.*, 2012). *C. glutamicum* lawn cells (100 μl) were mixed with 0.7% (v/v) top agar, then poured onto the MB plates. A total of 3.4 mg (20 μl of 0.25

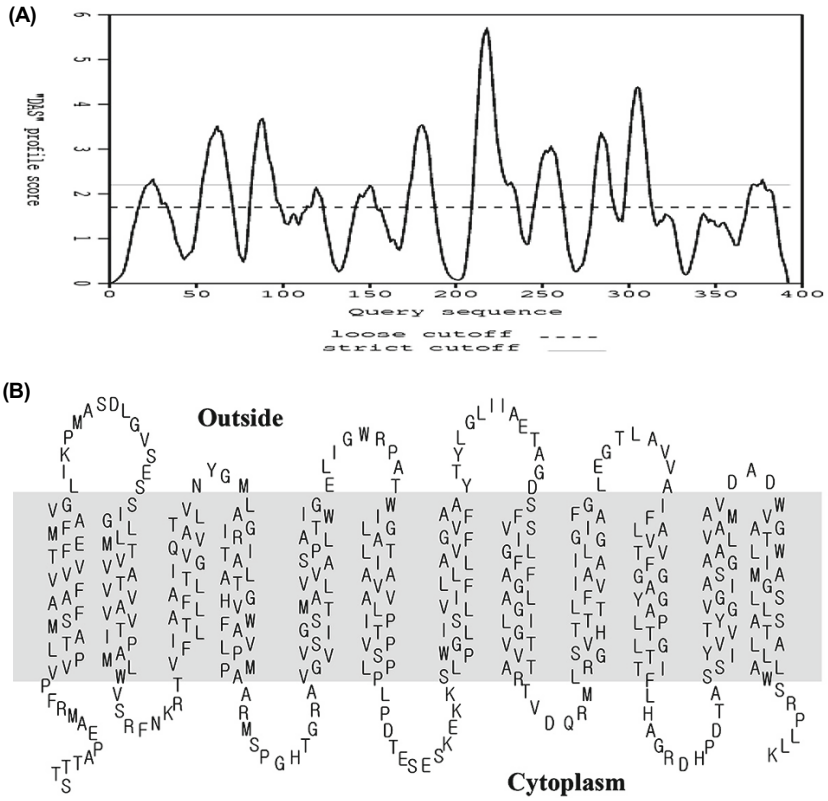

Fig. 1. **Prediction of transmembrane domains (A) and secondary structural model of CepA (B).**

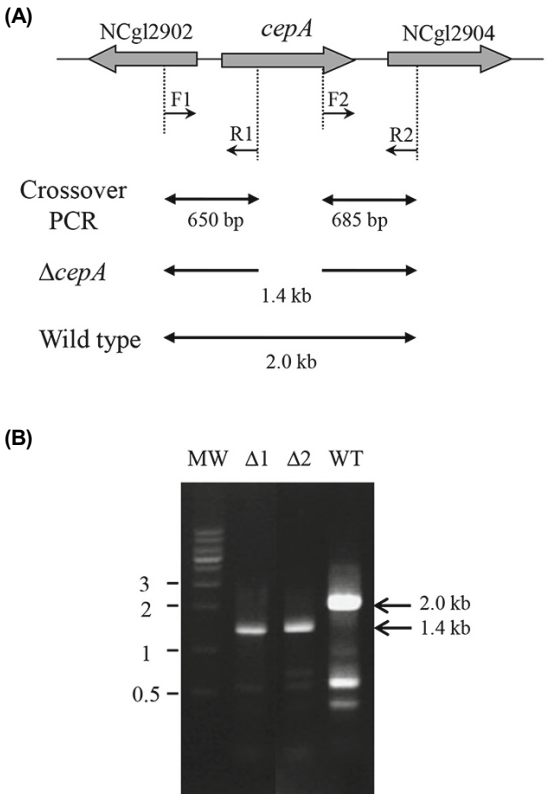

Fig. 2. Disruption of the *C. glutamicum cepA* gene by the crossover PCR method (A). Chromosomal deletion of the gene was identified in agarose gels by PCR amplification (B). To test for the deletion, primers of F1 and R2 were used. The predicted lengths of the amplified fragment are shown. Lanes: MW, marker (1 kb ladder); Δ1 and Δ2, *C. glutamicum* Δ*cepA* mutants; WT, wild type *C. glutamicum*.

M 6-mercapto purine or 0.5 M 6-mercapto guanine) was then applied to paper disks (6.0 mm, Whattman, UK), which were positioned on the plates. The plates were then incubated at 30°C for 24 h, until the complete formation of a clear-zone had occurred. MICs were determined in a MB broth at 30°C as described (Kim *et al.*, 2001). Crude extracts were prepared as described previously (Kim *et al.*, 2004). The chloramphenicol acetyltransferase (CAT, EC 2.3.1.28) assay was performed as described previously (Park *et al.*, 2004).

Results and Discussion

Identification of *cepA*

To identify *C. glutamicum* genes encoding proteins that are

Table 2. Chloramphenicol acetyltransferase activity of the constructed strains[a]

C. glutamicum strains	Plasmids	Phenotype	Activity (*cat*) (μmol/mg·min)
HL504	pSK1CAT	No promoter::*cat*	0.016
HL1037	pSL360	P_{180}::*cat*	11.2
HL1263	pSL461	P_{180}::*cepA*::*cat*	10.6

[a] Cells were grown on MB liquid medium to early stationary phase.

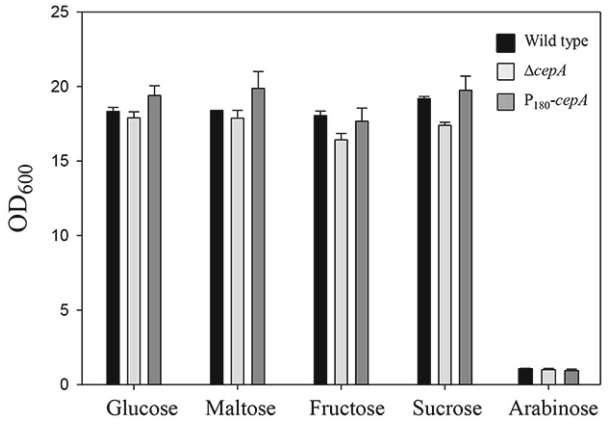

Fig. 3. Growth of *C. glutamicum* strains on various carbon sources. Wild-type (black), a *cepA*-deletion mutant (Δ*cepA*, light grey), or a *cepA*-over-expressing strain (P_{180}-*cepA*, dark grey) were grown on MCGC minimal medium supplemented with the indicated carbon sources. Final OD was measured after 24 h of incubation. Data represent three independent experiments.

functionally equivalent to the purine efflux proteins of *E. coli* [NepI (YicM)] and *B. subtilis* [PbuE (YdhL)], we carried out a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) of the *C. glutamicum* genome. First, we used the amino acid sequence of NepI and found several homologous proteins, which turned out to be mostly sugar transporters and major facilitator superfamily (MFS) proteins (Table 1). Among them, the NCgl2903-encoded protein showed highest similarity score (45%). The scores of the other screened proteins were lower than 40%. The ORF NCgl2903 consisted of 1,179 bp that encodes a putative arabinose efflux permease composed of 392 amino acids. Upon analysis with TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0) software, we were able to detect 12 transmembrane helixes in the NCgl2903-encoded protein, which is a typical feature of integral membrane transport proteins (Fig. 1B). Prediction of transmembrane alpha-helices using the Dense Alignment Surface method (Cserzo *et al.*, 1997) also agreed well with above data (Fig. 1A). When we used PbuE protein sequence to search for homologous proteins, NCgl0640- and

Table 3. Sensitivity of the *C. glutamicum* P_{180}-*cepA* strain to various purine analogues as determined by Kirby-Bauer tests

Class	Substances	Stock concentration[a]	Resistance[b]
Nucleosides	Adenosine	1 M	-
	Guanosine	1 M	-
	Inosine	1 M	-
Base analogues	2-aminopurine	1 M	-
	6-mercaptopurine	0.25 M	+
	6-mercaptoguanine	0.5 M	+
Nucleoside analogues	2-chloroadenosine	0.1 M	-
	7-methlyguanosine	0.1 M	-
Nucleotide analogues	7-methlyguanosine triphosphate	20 mM	-

[a] 20 μl of indicated stock solution was applied to paper disks.
[b] Resistance was determined by comparing the zone of inhibition to that of the wild type strain.

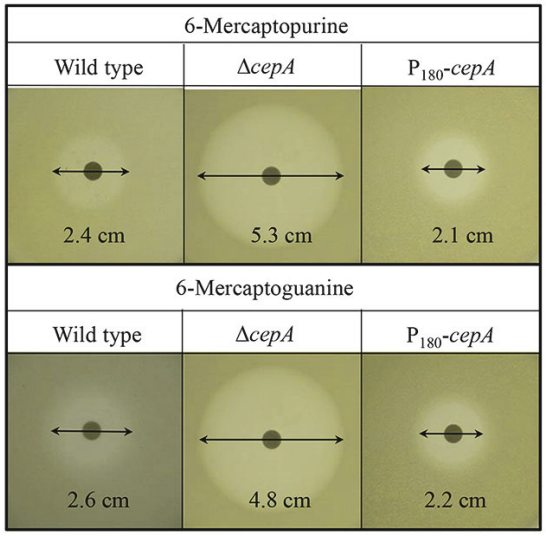

Fig. 4. Sensitivity of *C. glutamicum* strains to purine base analogs. A paper disc containing 3.4 mg of analogs was placed on each MB plate containing lawns of *C. glutamicum* cells (wild type, Δ*cepA*, or P_{180}-*cepA*) and incubated at 30°C for 24 h. Diameters are shown in centimeters. The data shown is from three independent experiments.

NCgl2903- encoded proteins showed highest similarity (43% and 42%, respectively). NCgl0640 was annotated to encode a sugar efflux permease. Based on the high similarity of NCgl2903- encoded protein to *E. coli* NepI and *B. subtilis* PbuE, we designated the ORF NCgl2903 as Corynebacterial efflux pump A (*cepA*) and selected it for in-depth analysis to reveal the function of the gene.

Deletion and amplification of *cepA*

To study the role of *cepA*, we constructed a *cepA*-deletion mutant strain (Δ*cepA*) by employing the technique developed by Schäefer *et al.* (1994). The chromosomal deletion of the internal 0.6 kb fragment of the gene was confirmed by PCR (Fig. 2). In addition, we also constructed a strain (P_{180}-*cepA*) that overexpresses the gene by employing the expression vector pSL360 which is known to achieve *tac* promoter-like overexpression of the cloned gene (Park *et al.*, 2004). We constructed the expression vector by inserting the *cepA* gene between the P_{180} promoter and the *cat* gene, thus creating transcriptional fusion of *cepA* with the *cat* gene (P_{180}-*cepA*-*cat*). As shown in Table 2, the chloramphenicol acetyltransferase (CAT) activity of the constructed strain (10.6 μmol/mg/min) was comparable to that of the strain carrying the parental vector P_{180}- *cat* (11.2 μmol/mg/min), suggesting successful overexpression of the *cepA* gene. No CAT activity was observed in the absence of the P_{180} promoter.

Because the *cepA* gene encoded a putative sugar efflux permease, we tested growth of the mutants on various carbon sources, such as glucose, maltose, fructose, and sucrose. On minimal medium, the final cell yield of the Δ*cepA* strain was slightly decreased, whereas that of the P_{180}-*cepA* strain was comparable or higher than that of the wild-type strain (Fig. 3). The cell yield differences between the Δ*cepA* and P_{180}-*cepA* strains were approximately 10%, suggesting that the gene does not play a critical role in sugar transport or in general cell physiology. No growth differences were observed when cultured on a complex medium (data not shown). Although the *cepA*-encoded protein showed a high degree of similarity with the *E. coli* arabinose transporter, *C. glutamicum* cells did not grow on media containing arabinose as the sole carbon source (Fig. 3).

Table 4. Sensitivity of *C. glutamicum* strains to various antibiotics

Class	Antibiotics	MIC[a] (μg/ml)	Resistance[b] (P_{180}-*cepA* strain)
β-Lactam	Ampicillin	15	+[c]
	Penicillin	0.8	-
Glycopeptide	Vancomycin	0.6	-
Antimycobacterial	Ethambutol	9	-
Quinolone	Nalidixic acid	200	+
Bacteriostatic	Chloramphenicol	6	-
	Tetracycline	20	-
Aminoglycoside	Kanamycin	0.4	-

[a] MIC (minimal inhibitory concentration) of tested antibiotics for the wild type *C. glutamicum* strain.
[b] Resistance of the *C. glutamicum* P_{180}-*cepA* strain to antibiotics relative to the wild type strain.
[c] Resistance was assessed by measuring MICs. See Table 5 for details.

Table 5. Susceptibilities of *C. glutamicum* strains to ampicillin and nalidixic acid

C. glutamicum strain	Relevant characteristic	MIC (μg/ml)[a]	
		Ampicillin	Nalidixic acid
AS019E12	Wild type	15	200
HL1264[b]	Δ*cepA*	7.5	175
HL1263[c]	P_{180}-*cepA*	20	250

[a] MICs were determined as described in the Materials and methods section.
[b] *C. glutamicum* AS019E12 with the *cepA* gene inactivated.
[c] *C. glutamicum* AS019E12 containing the plasmid P_{180}-*cepA* which overexpresses the *cepA* gene.

Resistance to purine base analogues and antibiotics

Knowing that the P_{180}-*cepA* strain showed enhanced growth as compared to the Δ*cepA* strain on the minimal glucose medium, we assumed that *cepA* might play an auxiliary role (e.g., function as an efflux pump for toxic chemicals). Because the *cepA*-encoded protein showed high similarity with the purine derivative exporters of *E. coli* and *B. subtilis*, we decided to monitor the responses after challenging the cells with purine analogues. As shown in Fig. 4, the Δ*cepA* strain showed increased sensitivity to 6-mercapto purine and 6-mercapto guanine, whereas the P_{180}-*cepA* strain showed decreased sensitivity as compared to the wild-type strain. Interestingly, the growth difference was not observed when the cells were challenged with 2-amino purine (Table 3) suggesting substrate specificity among purine analogues. When growth was tested in the presence of nucleoside analogues (2-chloroadenosine and 7-methylguanosine), and nucleotide analogues (7-methylguanosine triphosphate), no differences were observed (Table 3). Growth on nucleoside (such as adenosine, guanosine, or inosine)-containing media was idetical among the strains (data not shown). In *E. coli*, it is known that the amplification of YicM increases cell resistance to 6-mer-

captopurine as well as inosine, adenosine, and guanosine (Gronskiy *et al*., 2005). The *pbuE* gene from *B. subtilis* is also known to be involved in efflux of not only purine bases, but also purine ribonucleosides (Nygaard and Xaxild, 2005; Zakataeva *et al*., 2007). Collectively, these data, in conjunction with the growth data on different carbon sources suggest that the *cepA* gene could play a role in pumping out toxic chemicals such as purine analogues.

Assuming that the *cepA*-encoded protein might perform as an efflux pump, we next tested specificity by checking the mutant cell response to antibiotics. As shown in Table 4 and 5, cells overexpressing the *cepA* gene were notably more resistant to ampicillin and nalidixic acid than the wild-type and Δ*cepA* strains, suggesting that the protein has broad substrate specificity. We did not observe any difference in sensitivity to other antibiotics, including penicillin, vancomycin, ethambutol, chloramphenicol, tetracycline, and kanamycin (Table 4). Because the CepA protein showed similarity to other MFS proteins, we also tested the involvement of *cepA* in amino acid transport by challenging the cells with a growth-inhibiting amount of amino acids. However, no differences in growth between the wild-type and mutant cells were observed (data not shown).

Based on our findings that P_{180}-*cepA* cells are resistant to unrelated classes of antimicrobial compounds such as β-lactams (ampicillin), quinolones (nalidixic acid), and purine analogs, we presumed that the *cepA* gene product probably functions as of a multidrug-resistant efflux pump (Alekshun and Levy, 2007). It is known that one of the mechanisms of antimicrobial resistance to β-lactams or quinolones is active efflux (Li and Nikaido, 2004). Furthermore, as we observed in this study, overexpression of genes encoding components of efflux pumps is known to increase cell resistance to the respective inhibitors (Nikaido, 1996).

To date, only 1 drug-resistant efflux pump specific for lincosamides, has been described in *C. glutamicum* (Kim *et al*., 2001). Although, the CepA protein was identified on the basis of its similarity with *E. coli* and *B. subtilis* proteins, unlike these proteins, based on its substrate specificity, corynebacterial CepA may have novel functions and therefore requires further scientific attention. Furthermore, its contribution towards increasing cell growth, as seen in *cepA*-overexpressing cells (Fig. 3), may indicate that the protein has additional roles that are yet to be identified. Although we have not unveiled additional roles of the gene, based on its ability to boost cell growth, it may be useful in improving the industrial use of this organism.

Acknowledgements

This work was supported by a Korea University Grant to H.-S. Lee.

References

Alekshun, M.N. and Levy, S.B. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell* **128**, 1037–1050.

Cserzo, M., Wallin, E., Simon, I., von Heijne, G., and Elofsson, A. 1997. Prediction of transmembrane α-helices in prokaryotic membrane proteins: the dense alignment surface method. *Prot. Eng.* **10**, 673–676.

Eggeling, L. and Sahm, H. 2003. New ubiquitous translocators: amino acid export by *Corynebacterium glutamicum* and *Escherichia coli*. *Arch. Microbiol.* **180**, 155–160

Follettie, M.T., Peoples, O.P., Agoropoulou, C., and Sinskey, A.J. 1993. Gene structure and expression of the *Corynebacterium flavum* N13 *ask-asd* operon. *J. Bacteriol.* **175**, 4096–4103.

Gronskiy, S.V., Zakataeva, N.P., Vitushkina, M.V., Ptitsyn, L.R., Altman, I.B., Novikova, A.E., and Livshits, V.A. 2005. The *yicM* (*nepI*) gene of *Escherichia coli* encodes a major facilitator superfamily protein involved in efflux of purine ribonucleosides. *FEMS Microbiol. Lett.* **250**, 39–47.

Hwang, B.J., Yeom, H.J., Kim, Y., and Lee, H.S. 2002. *Corynebacterium glutamicum* utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis. *J. Bacteriol.* **184**, 1277–1286.

Ikeda, M. and Nakagawa, S. 2003. The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. *Appl. Microbiol. Biotechnol.* **62**, 99–109.

Johansen, L.E., Nygaard, P., Lassen, C., Agersø, Y., and Saxild, H.H. 2003. Definition of a second *Bacillus subtilis pur* regulon comprising the *pur* and *xpt-pbuX* operons plus *pbuG*, *nupG* (*yxjA*), and *pbuE* (*ydhL*). *J. Bacteriol.* **185**, 5200–5209.

Kalinowski, J., Bathe, B., Bartels, D., Bischoff, N., Bott, M., Burkovski, A., Dusch, N., Eggeling, L., Eikmanns, B.J., Gaigalat, L., and *et al.* 2003. The complete *Corynebacterium glutamicum* genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J. Biotechnol.* **104**, 5–25.

Kim, H.J., Kim, T.H., Kim, Y., and Lee, H.S. 2004. Identification and characterization of *glxR*, a gene involved in regulation of glyoxylate bypass in *Corynebacterium glutamicum*. *J. Bacteriol.* **186**, 3453–3460.

Kim, H.J., Kim, Y., Lee, M.S., and Lee, H.S. 2001. Gene *lmrB* of *Corynebacterium glutamicum* confers efflux-mediated resistance to lincomycin. *Mol. Cells* **12**, 112–116.

Lee, J.Y., Park, J.S., Kim, H.J., Kim, Y., and Lee, H.S. 2012. *Corynebacterium glutamicum whcB*, a stationary phase-specific regulatory gene. *FEMS Microbiol. Lett.* **327**, 103–109.

Leuchtenberger, W., Huthmacher, K., and Drauz, K. 2005. Biotechnological production of amino acids and derivatives: current status and prospects. *Appl. Microbiol. Biotechnol.* **69**, 1–8.

Li, X.Z. and Nikaido, H. 2004. Efflux-mediated drug resistance in bacteria. *Drugs* **64**, 159–204.

Marin, K. and Krämer, R. 2007. Amino acid transport systems in biotechnologically relevant bacteria, pp. 290–325. *In* Wendisch, V.F. (ed). Microbiol Monogr. Springer, Heidelberg, Germany.

Nikaido, H. 1996. Multidrug efflux pumps of Gram-negative bacteria. *J. Bacteriol.* **178**, 5853–5859.

Nygaard, P. and Saxild, H.H. 2005. The purine efflux pump PbuE in *Bacillus subtilis* modulates expression of the PurR and G-box (XptR) regulons by adjusting the purine base pool size. *J. Bacteriol.* **187**, 791–794.

Park, J.S., Lee, J.Y., Kim, H.J., Kim, E.S., Kim, P., Kim, Y., and Lee, H.S. 2012. The role of *Corynebacterium glutamicum spiA* gene in *whcA*-mediated oxidative stress gene regulation. *FEMS Microbiol. Lett.* **331**, 63–69.

Park, S.D., Lee, S.N., Park, I.H., Choi, J.S., Jeong, W.K., Kim, Y., and Lee, H.S. 2004. Isolation and characterization of transcriptional elements from *Corynebacterium glutamicum*. *J. Microbiol. Biotechnol.* **14**, 789–795.

Sambrook, J. and Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Schäfer, A., Tauch, A., Jager, W., Kalinowski, J., Thierbach, G., and Pühler, A. 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.

Sheremet, A.S., Gronskiy, S.V., Akhmadyshin, R.A., Novikova, A.E., Livshits, V.A., Shakulov, R.S., and Zakataeva, N.P. 2011. Enhancement of extracellular purine nucleoside accumulation by *Bacillus* strains through genetic modifications of genes involved in nucleoside export. *J. Ind. Microbiol. Biotechnol*. **38**, 65–70.

von der Osten, C.H., Barbas, C.F., Wong, C.H., and Sinskey, A.J. 1989. Molecular cloning, nucleotide sequence and fine-structural analysis of the *Corynebacterium glutamicum fda* gene: structural comparison of *C. glutamicum* fructose-1,6-biphosphate aldolase to class I and class II aldolases. *Mol. Microbiol*. **3**, 1625–1637.

Yukawa, H., Omumasaba, C.A., Nonaka, H., Kós, P., Okai, N., Suzuki, N., Suda, M., Tsuge, Y., Watanabe, J., Ikeda, Y., Vertès, A.A., and Inui, M. 2007. Comparative analysis of the *Corynebacterium glutamicum* group and complete genome sequence of strain R. *Microbiology* **153**, 1042–1058.

Zakataeva, N.P., Gronskiy, S.V., Sheremet, A.S., Kutukova, E.A., Novikova, A.E., and Livshits, V.A. 2007. A new function for the *Bacillus* PbuE purine base efflux pump: efflux of purine nucleosides. *Res. Microbiol*. **158**, 659–665.