Biotechnology and Bioprocess Engineering **2005**, 10: 510-515

Cloning and Sequencing of a Novel Glutaryl Acylase β-Subunit Gene of *Pseudomonas cepacia* BY21 from Bioinformatics

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Abstract *Pseudomonas cepacia* BY21 was found to produce glutaryl acylase that is capable of deacylating glutaryl-7-aminocephalosporanic acid (glutaryl-7-ACA) to 7-aminocephalosporanic acid (7-ACA), which is a starting material for semi-synthetic cephalosporin antibiotics. Amino acids of the reported glutaryl acylases from various *Pseudomonas* sp. strains show a high similarity (>93% identity). Thus, with the known nucleotide sequences of *Pseudomonas* glutaryl acylases in GenBank, PCR primers were designed to clone a glutaryl acylase gene from *P. cepacia* BY21. The unknown -subunit gene of glutaryl acylase from chromosomal DNA of *P. cepacia* BY21 was cloned successfully by PCR. The -subunit amino acids of *P. cepacia* BY21 acylase (GenBank accession number AY948547) were similar to those of *Pseudomonas diminuta* KAC-1 acylase except that Asn408 of *P. diminuta* KAC-1 acylase was changed to Leu408.

Keywords: 7-ACA, cephalosporin acylase, cephalosporin C, glutaryl acylase, DNA sequencing

INTRODUCTION

7-Aminocephalosporanic acid (7-ACA) is a starting material for semi-synthetic cephalosporin antibiotics. Industrial production of 7-ACA has been provided by the chemical deacylation of cephalosporin C (CPC). Since chemical processes require a low temperature, as well as the use of polluting chemicals, a two-step enzymatic conversion has been explored to overcome the defects of the chemical methods. The first step is catalyzed by D-amino acid oxidase to make glutaryl-7-ACA from CPC [1]. The second step is catalyzed by glutaryl acylase (EC 3.5.1.11) [2,3]. Several microorganisms of the genus *Bacillus* [4] and *Pseudomonas* [5-7] were reported to possess glutaryl acylase activity.

A single enzymatic conversion from cephalosporin C to 7-ACA has been studied by making a fusion protein of glutaryl acylase and D-amino acid oxidase [8] or by protein engineering of glutaryl acylase [9-13,19].

The *Pseudomonas* glutaryl acylase gene that encodes a 74-kDa polypeptide containing a signal peptide and an inactive 70-kDa precursor consists of α subunit (16-kDa), spacer peptides (9 amino acids: DPPDLADQG), and β(54-kDa) subunit [6,7,10,14]. The α-subunit of a *Pseudomonas* glutaryl acylase consists of 169 amino acid residues, while the β-subunit consists of 520 amino acid residues. The active glutaryl acylase is an $(\alpha\beta)_2$ heterotetramer complex. The crystal structure of a *Pseudomonas* glutaryl acylase (PDB accession number 1FM2) has been reported [15,16].

Amino acids of *Pseudomonas* glutaryl acylases are very similar (>93% identity) among *Pseudomonas* SY-77, *Pseudomonas* C427, *Pseudomonas* sp.130, and *Pseudomonas diminuta* KAC-1 [13]. Thus, it may be possible to clone another glutaryl acylase gene from a soil strain of *Pseudomonas* species by the use of PCR methodology. In this report, we describe the cloning and sequencing of a novel glutaryl acylase β-subunit gene from *Pseudomonas cepacia* BY21 by direct gene amplification.

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MATERIALS AND METHODS

Purification of Glutaryl Acylase

P. cepacia BY21 producing glutaryl-7-ACA acylase was fermented at 30°C for 40 h. The cells were disrupted by French press and centrifuged at 20,000 g. The supernatant was treated with ammonium sulfate up to 70% saturation. The precipitated proteins were dialyzed against phosphate buffer (100 mM, pH 7.5, 10% glycerol, 2 mM EDTA, 5 mM 2-mercaptoethanol, 1 mM PMSF) and applied to a DEAE-Sepharose FF column (20 cm × 5 cm). Proteins were eluted with a linear gradient of NaCl (300 mM) dissolved in 20 mM Tris-HCl buffer (pH 8.4). The acylase-active fractions were concentrated by ultrafiltration

Table 1. Primers for amplication of glutaryl aclylase gene

Name	Primer sequences	Glutaryl acylase gene
S-F	5′-ATG CTG AGA GTT CTG CAC C-3′	Signal: forward
α-F	5′-CTG GCC GAG CCG ACC TC-3′	α-subunit: forward
α-B	5′-AGG AGT TGG ATC CTT GAT CG-3′	α-subunit: backward
β-F	5′-CGA TCA AGG ATC CAA CTC CT-3′	β-subunit: forward
β-B	5′-TCA TGG CTT GAA GTT GAA GGG CGT G-3′	β-subunit: backward

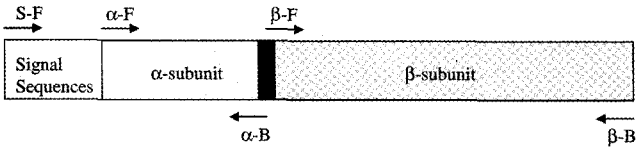

Fig. 1. Gene structure of *Pseudomonas* glutaryl acylase.

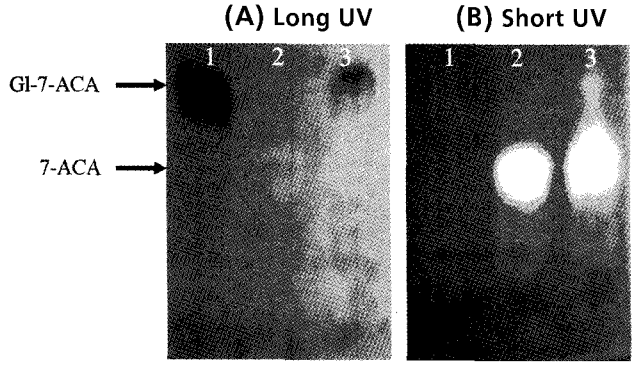

Fig. 2. TLC analysis of the product that is a mixture of glutaryl-7-ACA and glutaryl acylase purified from *P. cepacia* BY21. Lane 1: Glutaryl-7-ACA (standard), lane 2: 7-ACA (standard), and lane 3: the product.

and loaded to Sephadex G-100 gel chromatography (column 100 cm × 1.5 cm) and QAE-Sephadex A-50 chromatography (column 20 cm × 2.5 cm) for further purification.

Enzyme Assay

Substrate, glutaryl-7-aminocephalosporanic acid, was synthesized as described previously [2]. The reactants were analyzed using the colorimetric assay using 5% DAB [17] and TLC assay [3].

Molecular Cloning

Chromosomal DNA extracted from *P. cepacia* BY21 was used as a template DNA for glutaryl acylase gene amplification. PCR primers were designed as follows on the basis of the reported glutaryl acylase gene of *Pseudomonas* sp. SY-77 (GenBank accession number AF458663) (Table 1 and Fig. 1).

PCR was performed for 30 cycles with *pfu* DNA polymerase: preheating (95°C) 2 min, denaturation (95°C) 30 sec, annealing (57°C) 40 sec, elongation (72°C) 3 min, post-elongation (72°C) 10 min. The amplified single DNA fragment was cloned into a pGEM T-EASY cloning vector (Promega) and transferred to *E. coli* DH5α.

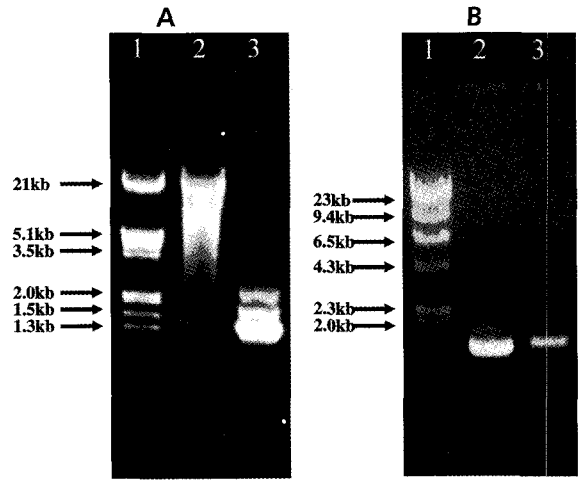

Fig. 3. DNA fragments amplified by PCR from the chromosomal DNA of *P. cepacia* BY21. (A) Lane 1: DNA marker, lane 2: chromosomal DNA, and lane 3: DNA fragments amplified with primers S-F and β-B. (B) Lane 1: DNA marker, lane 2: DNA fragments amplified with primers α-F and α-B, and lane 3: DNA fragments amplified with primers β-F and β-B.

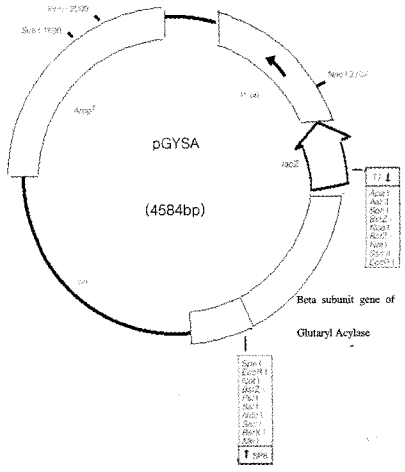

Fig. 4. Map of pGYSA which the pGEM cloning vector was inserted β-subunit gene of glutaryl acylase.

Table 2. The β-subunit gene of *P. cepacia* BY21 glutaryl acylase (GenBank accession number AY948547)

DNA:	TCCAACTCCTGGGCTGTGGCGCCGGGCAAGACGGCGAACGGGAACGCCCTGCTGTTGCAGAACCCG
+1:	S N S W A V A P G K T A N G N A L L L Q N P
DNA:	CACCTGTCCTGGACGACGGACTACTTCACCTACTACGAGGCGCATCTCGTCACGCCGGACTTCGAA
+1:	H L S W T T D Y F T Y Y E A H L V T P D F E
DNA:	ATCTATGGCGCGACCCAGATCGGCCTGCCGGTCATCCGCTTCGCCTTCAATCAGCGGATGGGCACC
+1:	I Y G A T Q I G L P V I R F A F N Q R M G I
DNA:	ATCAATACCGTCAACGGCATGGTGGGGGCCACCAACTATCGGCTGACGCTTCAGGACGGCGGCTAT
+1:	T N T V N G M V G A T N Y R L T L Q D G G Y
DNA:	CTGTATGACGGTCAGGTGCGGCCGTTCGAGCGGCGTCAGGCCTCGTATCGCCTGCGTCAGGCGGAC
+1:	L Y D G Q V R P F E R R Q A S Y R L R Q A D
DNA:	GGGTCGACGGTCGACAAGCCGTTGGAGATTCGCTCCAGCGTCCATGGCCCGGTCTTCGAGCGCGCG
+1:	G S T V D K P L E I R S S V H G P V F E R A
DNA:	GACGGCACGGCCGTCGCCGTTCGGGTCGCCGGTCTGGATCGGCCGGGCATGCTCGAGCAGTATTTC
+1:	D G T A V A V R V A G L D R P G M L E Q Y F
DNA:	GACATGATCACGGCGCACAGCTTCGACGACTACGAAGCCGCCATGGCGCGGATGCAGGTGCCGACC
+1:	D M I T A H S F D D Y E A A M A R M Q V P T
DNA:	TTCAACATCGTCTACGCCGACCGCGAAGGGACCATCAACTACAGCTTCAACGGAGTGGCGCCCAAA
+1:	F N I V Y A D R E G T I N Y S F N G V A P K
DNA:	CGGGCCGAGGGCGACATCGCCTTCTGGCAGGGGCTCGTGCCGGGCGATTCCTCGCGTTACCTGTGG
+1:	R A E G D I A F W Q G L V P G D S S R Y L W
DNA:	ACCGAGACCCACCCGCTGGATGATCTGCCGCGCGTCACCAATCCGCCGGGCGGCTTCGTGCAGAAC
+1:	T E T H P L D D L P R V T N P P G G F V Q N
DNA:	TCCAATGATCCGCCGTGGACGCCGACCTGGCCCGTCACCTACACGCCCAGGGACTTCCCCTCCTAT
+1:	S N D P P W T P T W P V T Y T P R D F P S Y
DNA:	CTGGCGCCCCAGACGCCGCACTCCCTGCGCGCACAACAAAGCGTGCGTCTGATGTCCGAGAACGAC
+1:	L A P Q T P H S L R A Q Q S V R L M S E N D
DNA:	GACCTGACGCTGGAGCGCTTCATGGCGCTACAGTTTAGCCATCGCGCCGTCATGGCCGACCGCACC
+1:	D L T L E R F M A L Q F S H R A V M A D R T
DNA:	TTGCCGGATCTGATCCCAGCGGCCCTGATAGACCCCGATCCCGAGGTCCAGGCGGCCGCGCGCCTG
+1:	L P D L I P A A L I D P D P E V Q A A A R L
DNA:	CTGGCGGCGTGGGATCGCGAGTTCACCAGCGACAGCCGCGCCGCCCTGCTGTTCGAGGAATGGGCG
+1:	L A A W D R E F T S D S R A A L L F E E W A
DNA:	CGTCTGTTCGCTGGCCAGAATTTCGCCGGCCAGGCCGGCTTCGCCACGCCCTGGTCGCTGGATAAG
+1:	R L F A G Q N F A G Q A G F A T P W S L D K
DNA:	CCGGTCAGCACCCCCTACGGTGTCCGCGACCCCAAGGCCGCCGTCGATCAACTGCGGACCGCTATC
+1:	P V S T P Y G V R D P K A A V D Q L R T A I
DNA:	GCCAACACCAAGCGCAAATACGGCGCGATCGACCGGCCGTTCGGCGACGCCTCGCGCATGATCCTG
+1:	A N T K R K Y G A I D R P F G D A S R M I L
DNA:	AACGACGTGAATGTTCCGGGCGCCGCCGGCTACGGCAACCTGGGTTCCTTCCGGGTCTTCACCTGG
+1:	N D V N V P G A A G Y G N L G S F R V F T W
DNA:	TCCGATCCCGACGAAAACGGGATTCGCACGCCCGTCCACGGCGAGACGTGGGTGGCGATGATCGAG
+1:	S D P D E N G I R T P V H G E T W V A M I E
DNA:	TTCTCCACGCCGGTGCGGGCCTATGGCCTGATGAGCTACGGCAACTCTCGCCAGCCGGGCACGACG
+1:	F S T P V R A Y G L M S Y G N S R Q P G T T
DNA:	CACTACAGCGATCAGATCGAACGCGTGTCGCGCGCCGACTTCCGCGAGCTGTTGCTGCGGCGAGAG
+1:	H Y S D Q I E R V S R A D F R E L L L R R E
DNA:	CAGGTCGAGGCCGCCGTCCAGGAACGCACGCCCTTCAACTTCAAGCCATGA
+1:	Q V E A A V Q E R T P F N F K P *

Sequencing

The cloned genes were sequenced by ABI 3100 system and compared with known genes by using Blast and ClustalW.

RESULTS AND DISCUSSION

P. cepacia BY21 was collected after fermentation and its cell extracts were purified by serial uses of DEAE-Sepharose FF, Sephadex G-100, and QAE-Sephadex A-

Table 3. Comparison of the β-subunit polypeptides of *Pseudomonas* glutaryl acylases reported in GenBank (accession number): *P. cepacia* BY21 (AY948547), *P. diminuta* KAC-1 (AF251710), *Pseudomonas* sp. 130 (AF085353), *Pseudomonas* sp. SY-77 (AF458663), and *Pseudomonas* sp. THA3 (AAP68798)

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BY21  SNSWAVAPGKTANGNALLLQNPHLSWTTDYFTYYEAHLVTPDFEIYGATQIGLPVIRFAF
KAC1  ....................................I...........................
#130  ....................................I...........................
SY77  ....................................I...........................
THA3  ....................................V...........................

BY21  NQRMGITNTVNGMVGATNYRLTLQDGGYLYDGQVRPFERRQASYRLRQADGSTVDKPLEI
KAC1  ..........................R.................S...................
#130  ..........................R.................T...................
SY77  ..........................P.................T...................
THA3  ..........................R.................S...................

BY21  1RSSVHGPVFERADGTAVAVRVAGLDRPGMLEQYFDMITAHSFDDYEAAMARMQVPTFNIV
KAC1  ..........................H.................M...................
#130  ..........................D.................L...................
SY77  ..........................D.................L...................
THA3  ..........................D.................M...................

BY21  YADREGTINYSFNGVAPKRAEGDIAFWQGLVPGDSSRYLWTETHPLDDLPRVTNPPGGFV
KAC1  ..........................N...................................
#130  ..........................L...................................
SY77  ..........................L...................................
THA3  ..........................L...................................

BY21  QNSNDPPWTPTWPVTYTPRDFPSYLAPQTPHSLRAQQSVRLMSENDDLTLERFMALQFSH
KAC1  ..........R.....................................F.....
#130  ..........K.....................................L.....
SY77  ..........K.....................................L.....
THA3  ..........K.....................................L.....

BY21  RAVMADRTLPDLIPAALIDPDPEVQAAARLLAAWDREFTSDSRAALLFEEWARLFAGQNF
KAC1  ..........................T...................................
#130  ..........................T...................................
SY77  ..........................T...................................
THA3  ..........................A...................................
BY21  AGQAGFATPWSLDKPVSTPYGVRDPKAAVDQLRTAIANTKRKYGAIDRPFGDASRMILND
KAC1  ..................P.........D.........I.........................
#130  ..................P.........D.........I.........................
SY77  ..................P.........D.........I.........................
THA3  ..................T.........A.........V.........................

BY21  VNVPGAAGYGNLGSFRVFTWSDPDENGIRTPVHGETWVAMIEFSTPVRAYGLMSYGNSRQ
KAC1  ..........F.....I.....................................
#130  ..........F.....V.....................................
SY77  ..........F.....V.....................................
THA3  ..........L.....V.....................................

BY21  PGTTHYSDQIERVSRADFRELLLRREQVEAAVQERTPFNFKP*
KAC1  ...............................................
#130  ...............................................
SY77  ...............................................
THA3  ...............................................
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50 chromatography columns. The purified glutaryl acylase was reacted with the substrate, glutaryl-7-aminocephalosporanic acid (Glutaryl-7-ACA) and the reactant was separated by TLC assay. When fluorescamin solution was sprayed onto the TLC plate, the fluorescent band corresponding to 7-ACA produced by glutaryl acylase was clearly shown (Fig. 2). The molecular size of the purified glutaryl acylase was approximately 70 kDa (data

not shown) when the protein was separated by use of nondenaturing polyacrylamide gel electrophoresis, indicating that this enzyme is very similar with other *Psedomonas* glutaryl acylases.

It was assumed that the glutaryl acylase gene of *P. cepacia* BY21 might be isolated by PCR amplification. Thus PCR primers were designed with one of the known *Pseudomonas* glutaryl acylase genes. Primers S-F and β-B were used to amplify the complete gene (2,163 bp) of glutaryl acylase. About three DNA fragments were amplified as shown in Fig. 3, suggesting that glutaryl acylase gene might be amplified with other genes. Therefore, each DNA fragment was cut out of the low-melting agarose gel and used as a DNA template for another PCR cycle with different primers. When primers α-F and α-B were applied to each DNA template, at least two DNA bands appeared, indicating that the primers were not specific to α-subunit gene. In contrast, when primers β-F and β-B were used, only a single DNA fragment appeared (Fig. 3). In an attempt to identify the amplified genes, DNA sequencing was performed with the amplified DNA fragments. Initial sequencing results of α-subunit gene part could not be read because other genes were possibly mixed together, while those of the β-subunit gene part matched well with the glutaryl acylase gene of *Pseudomonas* sp. SY-77, indicating that a single β-subunit gene was amplified.

Therefore, the amplified β-subunit gene was cloned into a pGEM T-EASY cloning vector (Promega, Fig. 4). The cloned β-subunit gene was sequenced completely (Table 2) and registered to the GenBank (accession number AY948547). When the β-subunit polypeptides of *P. cepacia* BY21 were compared with those of *Pseudomonas* sp. SY-77, 6 amino acid residues were different. When compared with other glutaryl acylases, such as the enzymes of *P. diminuta* KAC-1, *Pseudomonas* sp. 130, *Pseudomonas* sp. SY-77, *Pseudomonas* sp. THA3, the β-subunit acylase of *P. cepacia* BY21 was similar to that of *P. diminuta* KAC-1 except that only one amino acid, Leu408, was changed to Asn408 in *P. diminuta* KAC-1 acylase (Table 3). But other *Pseudomonas* glutaryl acylases have Leu408 rather than Asn408, indicating that this amino acid is not essential for the acylase activity.

It is known that Ser1 in the β-subunit is the catalytic residue for the autoproteolysis and catalytic reaction [14]. His-23 and Glu-455 in the β-subunit protein are also responsible for autoproteolysis [10]. Protein engineering of *Pseudomonas* glutaryl acylases was attributed to alteration of the catalytic efficiency as well as the substrate specificity. According to the structure-based mutagenesis, a triple mutant Q50βM/Y149αK/F177βG of *Pseudomonas* glutaryl acylase increased deacylation activity toward cephalosporin C up to 790% of the wild-type [18]. When the Asn-266 of glutaryl acylase was replaced with His and Met, the catalytic efficiency on adipyl-7-ADCA increased nearly 10 and 15 fold, respectively, without decreasing the catalytic efficiency on glutaryl-7-ACA [11,12]. Our finding of a new glutaryl acylase gene from *P. cepacia* BY21 would help more understanding of *Pseudomonas* acylases.

CONCLUSION

To isolate the unknown glutaryl acylase gene of *P. cepacia* BY21, PCR primers were designed from the published nucleotide sequences of *Pseudomonas* sp. SY-77. Glutaryl acylase consists of signal sequences, α-subunit peptides, spacer peptides, and β-subunit peptides. Gene amplification by PCR made it possible to successfully isolate the β-subunit gene of *P. cepacia* BY21. The β-subunit gene of *P. cepacia* BY21 was sequenced and reported to GenBank. The translated amino acids were compared with other known *Pseudomonas* glutaryl acylases by use of bioinformatic tools (BLAST and CLUSTAL W), showing that they were very similar to the β-subunit peptides of *P. diminuta* KAC-1.

Acknowledgement This work was kindly supported by the Korea Research Foundation (Grant No. KRF 2001-005-G20004).

REFERENCES

[1] Khang, Y.-H., I.-W. Kim, Y.-R. Hah, J.-H. Hwangbo, and K.-K. Kang (2003) Fusion protein of *Vitreoscilla* hemoglobin with D-amino acid oxidase enhances activity and stability of biocatalyst in the bioconversion process of cephalosporin C. *Biotechnol. Bioeng.* 82: 480-488.

[2] Franzosi, G., E. Battistel, I. Gagliardi, W. van der Goes (1995) Screening and characterization of microorganisms with glutaryl-7ADCA acylase activity. *Appl. Microbiol. Biotechnol.* 43: 508-513.

[3] Khang, Y.-H. and B.-H. Yoo (2000) Isolation and characterization of a novel soil strain, *Pseudomonas cepacia* BY21, with glutaryl-7-aminocephalosporanic acid acylase activity. *Biotechnol. Lett.* 22: 317-320.

[4] Aramori, I., M. Fukagawa, M. Tsumura, M. Iwami, H. Ono, H. Kojo, M. Kohsaka, Y. Ueda, and H. Imanaka (1991) Cloning and nucleotide sequencing of a novel 7β-(4-carboxybutanamido)cephalosporanic acid acylase gene of *Bacillus laterosporus* and its expression in *Escherichia coli* and *Bacillus subtilis*. *J. Bacteriol.* 173: 7848-7855.

[5] Lee, Y. H., T. S. Chang, H. J. Liu, and W. S. Chu (1998) An acidic glutaryl-7-aminocephalosporanic acid acylase from *Pseudomonas nitroreducens*. *Biotechnol. Appl. Biochem.* 28: 113-118.

[6] Lee, Y. S., H. W. Kim, K. B. Lee, and S. S. Park (2000) Involvement of arginine and tryptophan residues in catalytic activity of glutaryl 7-aminocephalosporanic acid acylase from *Pseudomonas* sp. strain GK16. *Biochim. Biophys. Acta* 1523: 123-127.

[7] Li, Y., J. Chen, W. Jiang, X. Mao, G. Zhao, and E. Wang (1999) *In vivo* post-translational processing and subunit reconstitution of cephalosporin acylase from *Pseudomonas* sp. 130. *Eur. J. Biochem.* 262: 713-719.

[8] Luo, H., Q. Li, H. Yu, and Z. Shen (2004) Construction and application of fusion proteins of D-amino acid oxidase and glutaryl-7-aminocephalosporanic acid acylase for direct bioconversion of cephalosporin C to 7-aminocephalosporanic acid. *Biotechnol. Lett.* 26: 939-945.

[9] Fritz-Wolf, K., K. P. Koller, G. Lange, A. Liesum, K. Sauber, H. Schreuder, W. Aretz, and W. Kabsch (2002) Structure-based prediction of modifications in glutarylamidase to allow single-step enzymatic production of 7-aminocephalosporanic acid from cephalosporin C. *Protein Sci.* 11: 92-103.

[10] Mao, X., W. Wang, W. Jiang, and G. P. Zhao (2004) His23beta and Glu455beta of the *Pseudomonas* sp. 130 glutaryl-7-aminocephalosporanic acid acylase are crucially important for efficient autoproteolysis and enzymatic catalysis. *Protein J.* 23: 197-204.

[11] Otten, L. G., C. F. Sio, J. Vrielink, R. H. Cool, and W. J. Quax (2002) Altering the substrate specificity of cephalosporin acylase by directed evolution of the Beta-subunit. *J. Biol. Chem.* 277: 42121-42127.

[12] Otten, L. G., C. F. Sio, A. M. van der Sloot, R. H. Cool, and W. J. Quax (2004) Mutational analysis of a key residue in the substrate specificity of a cephalosporin acylase. *Chembiochem* 5: 820-825.

[13] Sio, C. F., A. M. Riemens, J. van der Laan, R. M. D. Verhaert, and W. J. Quax (2002) Directed evolution of a glutaryl acylase into an adipyl acylase. *Eur. J. Biochem.* 269: 4495-4504.

[14] Lee, Y. S. and S. S. Park (1998) Two-step autocatalytic processing of the glutaryl 7-aminocephalosporanic acid acylase from *Pseudomonas* sp. strain GK16. *J. Bacteriol.* 180: 4576-4582.

[15] Kim, J. K., I. S. Yang, S. Rhee, Z. Dauter, Y. S. Lee, S. S. Park, and K. H. Kim (2003) Crystal structures of glutaryl 7-aminocephalosporanic acid acylase: insight into autoproteolytic activation. *Biochemistry* 42: 4084-4093.

[16] Kim, Y., K.-H. Yoon, Y.-H. Khang, S. Turley, and W. G. Hol (2000) The 2.0 Å crystal strucutre of cephalosporin acylase. *Structure* 8: 1059-1068.

[17] Shewale, J. G., K. K. Kumar, and G. R. Ambekar (1987) Evaluation of determination of 6-aminopenicillanic acid by *p*-dimethylaminobenzaldehyde. *Biotechnol. Tech.* 1: 69-72.

[18] Oh, B., M. Kim, J. Yoon, K. Chung, Y. Shin, D. Lee, and Y. Kim (2003) Deacylation activity of cephalosporin acylase to cephalosporin C is improved by changing the side-chain conformations of active-site residues. *Biochem. Biophys. Res. Commun.* 310: 19-27.

[19] Kim, J. H., J. S. Lim, and S. W. kim (2004) The improvement of cephalosporin C production by fed-batch cluture of *Cephalosporium acremonium* 25 using rice oil. *Biotechnol. Bioprocess Eng.* 9: 459-464.

[20] Gal, S. W., S. W. Lee, and Y. J. Choi (2002) Molecular cloning and characterization of 58 kDa chitinase gene from *Serratia marcescens* KCTC 2172. *Biotechnol. Bioprocess Eng.* 7: 38-42.

[21] Kim, O.-T., M.-Y. Kim, S.-J. Hwang, J.-C. Ahn, and B. Hwang (2005) Cloning and molecular analysis of cDNA encoding cycloartenol synthase from *Centella asiatica* (L.) urban. *Biotechnol. Bioprocess Eng.* 10: 16-22.

[Received March 17, 2005; accepted October 17, 2005]