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Characterization of Glutaryl 7-ACA Acylase from *Pseudomonas diminuta* KAC-1

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Abstract The glutaryl 7-aminocephalosporanic acid (glutaryl 7-ACA) acylase was purified from *Pseudomonas diminuta* KAC-1 cells isolated from soil, and characterized. The acylase was purified by procedures including ammonium sulfate fractionation and column chromatographies on DEAE-Sepharose, Phenyl-Sepharose, Q-Sepharose, and Superose 12H/R. The native acylase was found to be composed of two subunits with molecular masses of approximately 55 kDa and 17 kDa, respectively. The isoelectric point of the enzyme was 4.0. The specific activities of the purified acylase were 8.0 and 7.0 U/mg on glutaryl 7-ACA and glutaryl 7-aminodesacetoxy cephalosporanic acid (glutaryl 7-ADCA), respectively, and K_m values were 0.45 mM for glutaryl 7-ACA and 0.67 mM for glutaryl 7-ADCA. The enzyme had a pH optimum at 8.0 and a temperature optimum at 40 °C. The acylase catalyzed the synthesis of glutaryl 7-ACA from glutaric acid and 7-ACA as well as the hydrolysis of glutaryl 7-ACA, although the reaction rate of the synthesis was slower than that of the hydrolysis. In addition, it was found that the enzyme had a glutaryl transferase activity, thereby transferring the glutaryl group from one cephalosporin nucleus to another.

Key words: *Pseudomonas diminuta*, glutaryl 7-aminocephalosporanic acid acylase, N-terminal amino acid sequence

Cephalosporin acylase is an industrially important enzyme that converts cephalosporin C (CPC) to 7-ACA, which is a starting material for various clinically important semi-synthetic cephalosporin antibiotics. 7-ACA is prepared from CPC by cleavage of the aminoadipic side chain through chemical [11, 20] and enzymatic processes [9, 10, 26]. An enzymatic deacylation of cephalosporin C has been proposed for producing 7-ACA as an alternative route to conventional chemical processes. This two-step process of deacylation has been applied to the industrial production of 7-ACA. The first step includes oxidative deamination of the CPC aminoadipyl side chain using D-amino acid oxidase of *Trigonopsis variabilis* [25]. A keto derivative is transformed into glutaryl 7-ACA through a decarbonation reaction in the presence of hydrogen peroxide. In the second step, glutaryl 7-ACA is deacylated to form 7-ACA by the action of glutaryl 7-ACA acylase. Recently, the immobilized glutaryl 7-ACA acylase was also employed for production of 7-ACA [22].

Glutaryl 7-ACA acylases have already been purified and characterized from various strains belonging to the genus *Bacillus* and *Pseudomonas* [1, 2, 5, 6, 12, 13, 17, 18, 19, 21]. These enzymes can be divided into two types according to their substrate preference; namely, CPC acylase and glutaryl 7-ACA acylase. CPC acylase can deacylate both glutaryl 7-ACA and CPC to 7-ACA. The acylases of the SE83, A14, N176, and V12 strains belong to this category. The glutaryl 7-ACA acylases from *Pseudomonas* sp. strains, including GK-16, C427, BL072, and 130, have been shown to efficiently remove the acyl side chain of glutaryl 7-ACA to give 7-ACA, however, these enzymes are not active on CPC. Almost all the glutaryl 7-ACA acylases characterized so far are heterodimers derived from a single polypeptide precursor following a proteolytic cleavage after its synthesis. The two fragments are then assembled to form the mature protein. These enzymes are composed of large and small subunits with a molecular mass of 60,000–80,000 Da [24].

We earlier reported *Pseudomonas diminuta* KAC-1 strain as a glutaryl 7-ACA acylase producer [14], and the present paper describes the purification and characterization of the glutaryl 7-ACA acylase from *P. diminuta* KAC-1.

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MATERIALS AND METHODS

Chemicals

7-ACA was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Glutaryl 7-ACA used as the substrate in the acylase reaction was kindly supplied by Chong Kun Dang Co. (Seoul, Korea). Glutaric acid, acetonitrile, and para-dimethylaminobenzaldehyde were obtained from Merck (Frankfurt, Germany).

Organism and Growth Conditions

P. diminuta KAC-1, isolated from soil, was used as the glutaryl 7-ACA acylase producer [14]. Strain KAC-1 was grown in a batch culture using a 5-l fermenter at 25°C. The complex medium contained 20 g of glucose, 3 g of beef extract, 5 g of peptone, 5 g of monosodium glutamate, and 1 g of glutaric acid per 1-l water, and the pH was adjusted to 9.0. The agitation speed and aeration rate were maintained at 200 rpm and 2.0 vvm, respectively [15, 23].

Enzyme Assays

The glutaryl 7-ACA acylase was assayed using the colorimetric method described by Balasingham [3]. A reaction mixture (0.5 ml in 100 mM Tris-HCl buffer, pH 8.0) containing 10 mM glutaryl 7-ACA was incubated at 37°C. After adding glutaryl 7-ACA, the reaction was stopped by the addition of 3 ml of a mixture of 20% acetic acid and 0.05 M NaOH (2:1). The reaction mixture was then centrifuged and 0.5 ml para-dimethylaminobenzaldehyde (0.5% w/v in methanol) was added to the supernatant. The 7-ACA released was determined by measuring absorbance at 415 nm. One unit of acylase activity was defined as the quantity of enzyme that produced 1 μmole of 7-ACA per min under the defined conditions.

The assay of the reaction components including 7-ACA, glutaryl 7-ACA, glutaryl 7-ADCA, and 7-ADCA was performed using an HPLC (Millipore, Milford, MA, U.S.A.). The reaction mixtures were injected onto a C18 reverse-phase HPLC column with a mobile phase consisting of 20 mM ammonium acetate (pH 5.0) and acetonitrile (98:2), and detected at 250 nm. The protein concentration was determined by the Bradford method [7] using bovine serum albumin as the standard protein. The N-terminal amino acid sequence of purified enzyme was determined using the 470A protein sequencer (Applied Biosystems, Foster City, CA, U.S.A.).

Enzyme Purification

The *Pseudomonas* sp. KAC-1 cells were suspended in 0.1 M Tris-HCl (pH 8.0), and disrupted by ultrasonication. The cell debris was removed by centrifugation, then the supernatant was adjusted to 35% saturation with ammonium sulfate. After removing the precipitate, the supernatant was brought to 60% saturation with solid ammonium sulfate. The resulting precipitate was collected by centrifugation, and dissolved in a 100 mM Tris-HCl buffer (pH 8.0). The enzyme solution, dialyzed against the same buffer, was loaded onto a DEAE-Sepharose column equilibrated with the same buffer. After loading, the acylase was eluted with a 0–0.5 M linear gradient of NaCl in 100 mM Tris-HCl buffer (pH 8.0). The eluate fractions showing acylase activity were pooled and dialyzed against 100 mM Tris-HCl buffer (pH 8.0) containing 0.3 M ammonium sulfate, followed by phenyl-Sepharose column chromatography. The acylase was eluted with a 0.3 to 0 M linear gradient of ammonium sulfate. The fractions containing acylase were pooled and dialyzed against a 25 mM Tris-HCl buffer (pH 8.0). The sample was loaded onto a Q-Sepharose column and eluted with a linear NaCl gradient (0 to 0.5 M). The active fractions were concentrated with ultrafiltration and loaded onto a Superose 12 column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with 50 mM Tris-HCl buffer (pH 8.0) and 0.15 M NaCl. The purified glutaryl 7-ACA acylase was concentrated by ultrafiltration and analyzed by SDS-PAGE. The molecular mass of the purified enzyme was determined by SDS-PAGE and FPLC using a Superose 12 HR 10/30 column.

Determination of Isoelectric Point

The isoelectric focusing was performed using a Phastsystem with PhastGel IEF 3–9 media (Pharmacia Biotech, Uppsala, Sweden). After gel running, the protein in the gel was fixed by soaking the gel in 20% TCA, and the gel was then stained with Coomassie Brilliant Blue and destained.

RESULTS AND DISCUSSION

Purification and Physical Characterization of KAC-1 Glutaryl 7-ACA Acylase

Glutaryl 7-ACA acylases have been reported to localize in the periplasm or cytoplasm of *Pseudomonas* strains [1, 4, 6, 12, 16]. In the KAC-1 strain, glutaryl 7-ACA acylase was found in the periplasm and cytoplasm in different proportions depending on the growing conditions (data not shown). Therefore, the total cell extract was used for the acylase purification. The procedures used for purifying the glutaryl 7-ACA acylase from the cell extract are summarized in Table 1. The KAC-1 cells were disrupted by ultrasonication and centrifuged. The cell-free extract was then treated with 35–60% ammonium sulfate for acylase precipitation. Purified acylase was obtained after chromatographies on DEAE-Sepharose, Phenyl-Sepharose, Q-Sepharose, and Superose. Since the KAC-1 strain produces β-lactamase, which can degrade β-lactam compounds including glutaryl 7-ACA and 7-ACA, the β-lactamase has to be removed for an accurate assay of the acylase activity. Accordingly, this enzyme was completely washed out by DEAE-Sepharose column chromatography.

Table 1. Summary of the purification steps of KAC-1 acylase.

Purification steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Cell-free extract*	1,073	18.5	0.02	1.0	100
Ammonium sulfate*	350	11.7	0.03	1.5	63
DEAE Sepharose	74	8.1	0.11	5.5	44
Phenyl Sepharose	4.3	5.3	1.23	61.5	29
Q Sepharose	2.2	5.1	2.32	116.0	27
Superose 12	0.2	1.3	6.63	331.0	7

*The enzyme reaction was performed in the presence of clavuranic acid, which is a β-lactamase inhibitor.

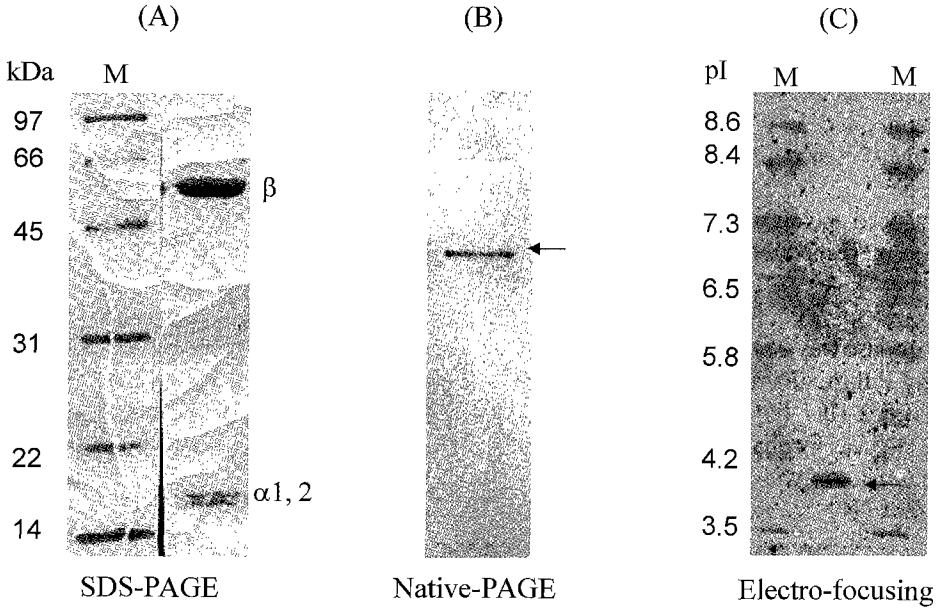

Fig. 1. Molecular properties of purified KAC-1 acylase.
(A) SDS-PAGE of KAC-1 acylase. (B) Native-PAGE of KAC-1 acylase. (C) Isoelectro focusing gel of KAC-1 acylase. The arrow indicates the purified KAC-1 acylase and its subunits.

The purified acylase gave three subunits on SDS-PAGE (Fig. 1A), which were a β subunit of 55 kDa and two α subunits, named α1 and α2, with a similar molecular mass of approximately 17 kDa. This three-subunit composition of KAC-1 acylase is not common for the glutaryl 7-ACA acylases of *Pseudomonas* sp., which are normally heterodimers consisting of α and β subunits [1, 4, 6, 12, 16, 17]. The molecular mass of the native KAC-1 acylase was also estimated to be 74 kDa by gel permeation chromatography, thereby suggesting that KAC-1 acylase is also a heterodimer with α and β subunits.

To investigate the α1 and α2 subunits of KAC-1 acylase, their N-terminal amino acid sequences were determined (Table 2). The results revealed that the two subunits were identical, while the α1 subunit had 3 amino acid residues more than the α2 subunit in the N-terminus. It is assumed that the difference in the N-terminal residues of the α1 and α2 subunits of KAC-1 acylase was caused by a signal peptidase during the post-translational process. Penicillin G amidohydrolase [8] and BL072 acylase [6] have also been reported to be processed into several types of α subunit by signal peptidase.

Table 2. Comparison of N-terminal amino acid sequences of KAC-1 acylase subunits with others.

Bacterial strains	N-terminal sequence of α-subunit	N-terminal sequence of β-subunit	Ref.
Pseudomonas sp. C427	LAEPTSTPQAPIAAYKPRS	SNSWAVAPGKTANGNALLL	[13]
Pseudomonas sp. GK16	EPTSTPQAPIAAYKPRS	SNSWAVAPGKTANGNALLL	[12]
Pseudomonas sp. 130	LAEPTSTPQAPIAAYKPRS	SNSWAVAPGKTANGNALLL	[15]
Pseudomonas sp. KAC-1 α1	EPTSTPQAPIAAYKPRS	SNSWAVAPGKTANGNALLL	this work
Pseudomonas sp. KAC-1 α2	TPQAPIAAYKPRS		this work

The isoelectric point of the acylase was determined with a Phast isoelectric focusing gel (Fig. 1C). Only one protein band with a pI value of 4.0 was detected in the IEF gel, suggesting that the two forms of KAC-1 acylase, composed of α1β and α2β, have the same conformation. The pI values of reported acylases from strains BL072, *P. nitroreducens* [16], and NCIMB 40474 [4] are 8, 5.3, and 4.4, respectively.

Biochemical Characterization of the KAC-1 Glutaryl 7-ACA Acylase

The substrate specificity of cephalosporin acylase is regarded as important for the industrial production of 7-ACA. KAC-1 acylase was found to deacylate glutaryl 7-ACA and glutaryl 7-ADCA, yet it was not active on CPC and other cephalosporin compounds. The specific activities of the purified KAC-1 acylase for glutaryl 7-ACA and glutaryl 7-ADCA were 8.0 and 7.0 U/mg of protein, and the K_m values were 0.45 mM and 0.67 mM, respectively. The V_{max} values of the enzyme were 8.0 μmol/mg protein/min and 7.0 μmol/mg protein/min for the hydrolysis of glutaryl 7-ACA and glutaryl 7-ADCA, respectively (Fig. 2). Besides its acylase activity, KAC-1 acylase was found to exhibit γ-glutamyl transpeptidase (γ-GT) activity. This enzyme also hydrolyzed several amino acid-para-nitroanilide (pNA) compounds, including Val-, Met-, Leu-, Gly-, Glu-, and Arg-pNA, except for Phe-pNA. It has been reported that some glutaryl 7-ACA acylases show a similarity to γ-GT according to the amino acid sequence and enzyme activity [6, 16]. These results indicate that the substrate specificities of KAC-1 acylase were affected by the side chain of the substrates rather than the lactam ring.

The optimal temperature and pH for the glutaryl 7-ACA hydrolysis reaction of KAC-1 acylase were determined. The optimal temperature was 40°C, and the optimal pH was 8.0. Since reaction substrates and products such as β-lactam antibiotics are unstable at high temperature and pH, information on the reaction properties of KAC-1 acylase are thus important for the enzymatic production of 7-ACA compounds.

The glutaryl 7-ACA hydrolysis reaction of the acylase was influenced by the reaction product, 7-ACA, and glutaric acid. The hydrolysis reactions of the acylases isolated from strains A14 and N176 are dramatically inhibited by 5 mM concentration of 7-ACA [1]. The reaction rate of KAC-1 acylase was maintained over 90% in the presence of either 6 mM glutaric acid and 6 mM 7-ACA (Fig. 3). However, the reverse reaction (condensation) of glutaryl 7-ACA acylase was observed when the concentration of both glutaric acid and 7-ACA were 20 mM (Fig. 4A).

In addition, the transferase activity of KAC-1 acylase was detected when the enzyme reaction was carried out with 7-ACA and glutaryl 7-ADCA as the reaction substrates. During the reaction, the concentrations of the starting materials decreased and glutaryl 7-ACA was produced, as shown in Fig. 4B. The reaction rate was 20-fold faster than the condensation reaction rate (Fig. 4A) of 7-ACA and glutaric acid to produce glutaryl 7-ACA. This result indicates that KAC-1 acylase contains glutaryl transferase activity which can transfer the glutaryl residue from one cephalosporin compound to another.

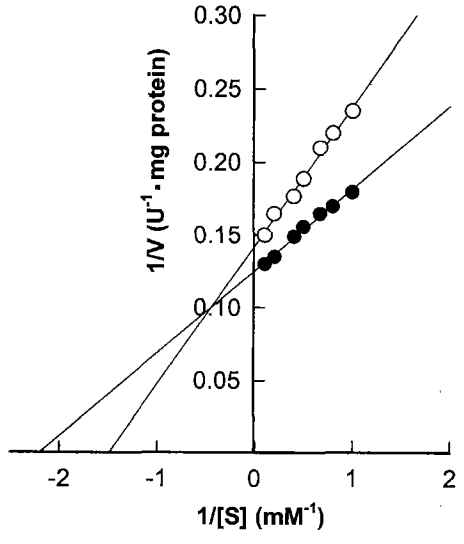

Fig. 2. Lineweaver-Burke plot of various concentrations of glutaryl 7-ACA (●) and glutaryl 7-ADCA (○) relative to the KAC-1 acylase reaction rate.
The reaction mixture contained KAC-1 acylase and various concentrations of glutaryl 7-ACA and glutaryl 7-ADCA. After the reaction, an HPLC assay was performed as described in Materials and Methods.

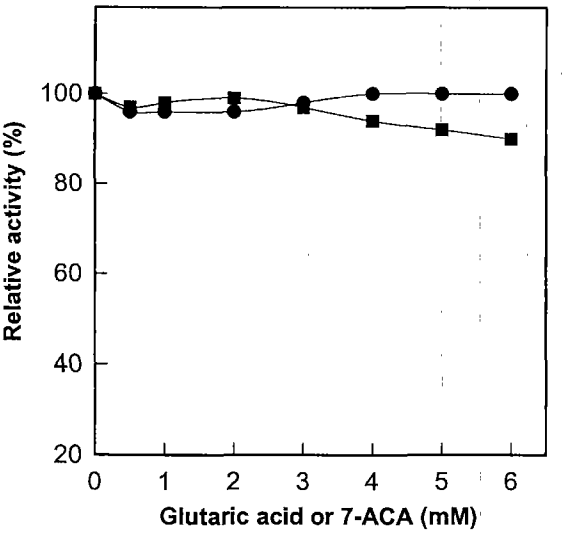

Fig. 3. Effects of reaction products on the activity of KAC-1 acylase.
Glutaric acid (●) and 7-ACA (■) were added to the reaction mixture at various concentrations, respectively.

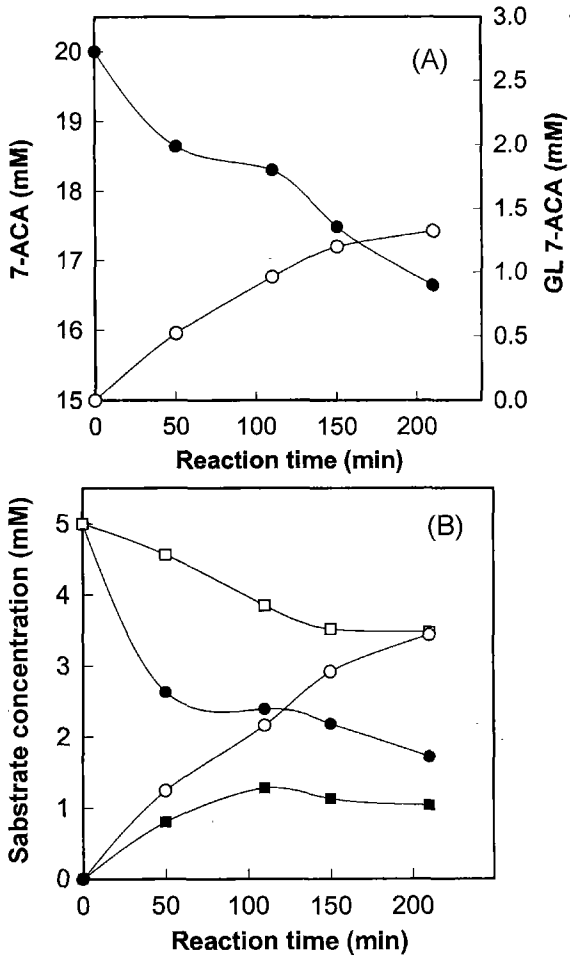

Fig. 4. Glutaryl 7-ACA synthesis activity and glutaryl transferase activity of KAC-1 acylase.

The reaction mixture for glutaryl 7-ACA synthesis activity (A) of glutaryl 7-ACA acylase contained 20 mM glutaric acid and 20 mM 7-ACA. The residual 7-ACA (●) and newly formed glutaryl 7-ACA (○) were determined using the HPLC method. The reaction mixture contained 20 mM glutaryl 7-ADCA and 20 mM 7-ACA as the substrates for the transferase activity of the acylase (B). The residual glutaryl 7-ADCA (○), 7-ADCA (□), glutaryl 7-ACA (■), and 7-ACA (●) were determined by an HPLC.

Comparison of N-Terminal Amino Acid Sequence of KAC-1 Glutaryl 7-ACA Acylase with Other Acylase Sequences

Cephalosporin acylases have been grouped into five classes (I-V) on the basis of their gene structure, molecular mass, and enzymatic properties [18]. The N-terminal amino acid sequences of the KAC-1 acylase subunits were compared with other cephalosporin acylases. It was found that the N-terminal amino acid sequence of the β subunit was the same as those of the β subunits from GK16, 130, and C427 of *Pseudomonas* sp. which belong to class I cephalosporin acylases (Table 2). The first N-terminal amino acid residues of the α subunits were different due to different signal peptidase cleavages. Nevertheless, their N-terminal sequences were identical, therefore, KAC-1 acylase should belong to the class I.

Acknowlegment

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REFERENCE

1. Aramori, I., M. Fukagawa, M. Tsumura, M. Iwami, H. Kojo, H. Ono, M. Kohsaka, Y. Ueda, M. Kohsaka, and H. Imanaka. 1992. Comparative characterization of new glutaryl 7-ACA and cephalosporin C acylases. *J. Ferment. Bioeng.* **73:** 185–192.
2. Aramori, I., M. Fukagawa, M. Tsumura, M. Iwami, H. Ono, H. Kojo, M. Kohsaka, Y. Udea, and H. Imanaka. 1991. Cloning and nucleotide sequence of a novel 7β-(4-carboxybutanamido) cephalosporanic acid acylase gene of *Bacillus laterosoporus* and its expression in *Escherichia coli* and *Bacillus subtilis*. *J. Bacteriol.* **173:** 7848–7855.
3. Balasingham, K., D. Warburton, P. Dunnill, and M. Lilly. 1972. The isolation and kinetics of penicillin amidase from *Escherichia coli*. *Biochim. Biophys. Acta* **276:** 250–256.
4. Battistel, E., D. Bianchi, R. Bortolo, and L. Bonoldi. 1998. Purification and stability of glutaryl 7-ACA acylase from *Pseudomonas* sp. *Appl. Biochem. Biotechnol.* **69:** 53–67.
5. Binder, R., G. K. Numata, D. A. Lowe, T. Murakami, and J. L. Brown. 1993. Isolation and characterization of a *Pseudomonas* strain producing glutaryl 7-aminocephalosporanic acid acylase. *Appl. Environ. Microbiol.* **59:** 3321–3326.
6. Binder, R., J. L. Brown, and G. Romancik. 1994. Biochemical characterization of a glutaryl 7-aminocephalosporanic acid acylase from *Pseudomonas* strain BL072. *Appl. Environ. Microbiol.* **60:** 1805–1809.
7. 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.* **7:** 248–254.
8. Burtscher, H. and G. Schumacher. 1992. Reconstitution *in vivo* of penicillin G acylase activity from separately expressed subunits. *Eur. J. Biochem.* **205:** 77–83.
9. Choi, D.-H., Y.-D. Kim, I.-S. Chung, S.-H. Lee, S.-M. Kang, T.-J. Kwon, and K.-S. Han. 2000. Gene cloning and expression of cephalosporin-C deacetylase from *Bacillus* sp. KCCM10143. *J. Microbiol. Biotechnol.* **10:** 221–226.
10. Demain, A. L., R. B. Walton, J. F. Newkirik, and I. M. Miller. 1963. Microbial degradation of cephalosporin C. *Nature* **199:** 909–910.
11. Fechtig, B., H. Peter, H. Bickel, and E. Wischer. 1986. Ber die herstellung von 7-aminocephalosporans ure. *Helv. Chim. Acta* **51:** 1108–1119.
12. Ichikawa, S., Y. Murai, S. Yamamoto, Y. Shibuya, T. Fujii, K. Komatsu, and R. Kodaira. 1981. The isolation and properties of *Pseudomonas* mutants with an enhanced productivity of 7β-(4-carboxybutanamido) cephalosporanic acid acylase. *Agric. Biol. Chem.* **45:** 2225–2229.

13. Ishii, Y., Y. Saito, T. Fujimura, T. Isoga, H. Kojo, M. Yamashita, M. Niwa, and M. Kohsaka. 1994. A novel 7-β-(4-carboxybutanamido)-cephalosporanic acid acylase isolated from *Pseudomonas* strain C427 and its high-level production in *Escherichia coli*. *J. Ferment. Bioeng.* **77:** 591–597.
14. Kim, D. W., S. M. Kang, and K.-H. Yoon. 1999. Isolation of novel *Pseudomonas diminuta* KAC-1 strain producing glutaryl 7-aminocephalosporanic acid acylase. *J. Microbiol.* **37:** 200–205.
15. Lee, E. G., E.-H. Park, and H.-H. Hyun. 2000. Purification and characterization of two alkaline proteases produced by *Pseudomonas* sp. BK7. *J. Microbiol. Biotechnol.* **10:** 677–684.
16. Lee, Y. H., T. S. Chang, H. J. Liu, and W. S. Chu. 1998. An acidic glutaryl 7-aminocephalosporanic acid acylase from *Pseudomonas nitroreducens*. *Biotech. Appl. Biochem.* **28:** 113–118.
17. Lee, Y.-S., H.-C. Yang, and S.-S. Park. 1996. Cloning and characterization of GL 7-ACA acylase gene from *Pseudomonas* sp. GK16. *J. Microbiol. Biotechnol.* **6:** 375–380.
18. 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:** 710–713.
19. Matsuda, A., K. Matsuyama, K. Yamamoto, S. Ichikawa, and K. Komatsu. 1987. Cloning and characterization of the genes for two distinct cephalosporin acylase from a *Pseudomonas* strain. *J. Bacteriol.* **169:** 5815–5820.
20. Morin, R. B., B. G. Jackson, E. H. Flynn, R. W. Roeske, and S. L. Andrews. 1969. Chemistry of cephalosporin antibiotics. XIV. Reaction of cephalosporin C with nitrosyl chloride. *J. Amer. Chem. Soc.* **91:** 1396–1399.
21. Shibuya, Y., K. Matsumoto, and T. Fujii. 1981. Isolation and properties of 7β-(4-carboxybutanamido)cephalosporanic acid acylase-producing bacteria. *Agric. Biol. Chem.* **45:** 1561–1567.
22. Shin, H.-J., S.-G. Lee, W.-S. Lee, and K.-H. Yoon. 1996. Enzymatic conversion of glutaryl 7-aminocephalosporanic acid to 7-aminocephalosporanic acid with an immobilized glutaryl 7-aminocephalosporanic acid acylase. *J. Microbiol. Biotechnol.* **6:** 336–339.
23. Shinde, M., C.-K. Kim, and T. B. Karegoudar. 1999. Production of salicylic acid from naphthalene by immobilized *Pseudomonas* sp. strain NGK1. *J. Microbiol. Biotechnol.* **9:** 482–487.
24. Sudhakaran, V. K., B. S. Deshpande, S. S. Ambedkar, and J. G. Shewale. 1991. Molecular aspects of penicillin and cephalosporin acylases. *Process Biochem.* **27:** 131–143.
25. Szwajcer-Dey, E., J. R. Miller, S. Kovacevic, and K. Mosbach. 1990. Characterization of a D-amino acid oxidase with high activity against cephalosporin C from the yeast *Trigonopsis variabilis*. *Biochem. Int.* **20:** 1169–1178.
26. Walton, R. B. 1964. Search for microorganisms producing cephalosporin C amidase. *Dev. Industrial Microbiol.* **5:** 349–363.