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An Efficient Method for the Release of Recombinant Penicillin G Amidase from the *Escherichia coli* Periplasm

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In this study, we report on a simple, efficient method for obtaining penicillin G amidase (PGA) from recombinant *Escherichia coli* using a formulation mixed with detergent and lysozyme. Research was conducted on the extraction efficiency of PGA from the periplasmic space in cells in terms of the type of detergent, detergent concentration, pH, reaction time, and temperature of permeabilization. The extraction yield of PGA in the formulated surfactant/lysozyme treatment was increased by approximately (55-65 U/ml) in comparison with that in the single surfactant treatment. The released PGA solution was concentrated and exchanged with buffer using an ultrafiltration (U/F) system. The yields of diatomite filtration, membrane filtration (M/F), and U/F were 69.7%, 93.8%, and 77.3%, respectively. A total of 212 KU of PGA was recovered. At the 25-L culture scale, the overall yield of extraction using the mixed surfactant/lysozyme method was 49.2%. The specific activity of extracted PGA was 11 U/mg in protein. The concentrated PGA solution was immobilized on microporous silica beads without further purification of PGA. The total immobilization yield of PGA on the resin was 48.7%, while the enzyme activity was 101 U/g. The immobilized PGA was successfully used to produce 6-APA from penicillin G. Our results indicated that a simple extraction method from periplasmic space in *E. coli* may be used for the commercial scale production of β-lactam antibiotics using immobilized PGA.

Key words : Detergent, immobilization, lysozyme, penicillin G amidase, permeabilization

Introduction

Penicillin G amidase (PGA; EC 3.5.1.11), benzylpenicillin-amidohydrolase, is heterodimer with small subunit (M.W. =24,000) and large subunit (M.W. = 65,000). PGA is one of the key enzyme in the antimicrobial industry for production of β-lactam antibiotics [8]. PGA gene was cloned and expressed in *E.coli* periplasm. The PGA in *E. coli* ATCC 11105 is secreted into the periplasm after removing signal sequences and becomes heterodimer which composed of two subunits, small subunit (24 kDa) and large subunit (65 kDa). In this study, the PGA gene was obtained from *E. coli* ATCC 11105 using PCR (polymerase chain reaction) technique. The active PGA was successfully secreated into periplasm in *E. coli* BL21 (DE3) harboring pET-pga plasmid [14]. The cytoplasmic expression is the common method for the production of recombinant proteins in *E.coli*. However, intracellular expression has various disadvantages such as insoluble inclusion bodies formation and protein misfolding. The expression of interested protein within the *E.coli*'s periplasmic space eliminates these demerits and simplifies protein purification [4]. Therefore, recombinant proteins can be expressed into the periplasmic space in *E.coli* [6, 14]. Also, the production of recombinant protein in *E.coli*'s periplasmic space protects the protein from proteolytic digestion [3]. The periplasmic space contains 7 out of the 25 known cellular proteases and consists only 4~8% of the total cellular protein. If the recovery of a recombinant protein from the periplasm can be achieved in a small volume without contamination by intracellular proteins, subsequent purification steps are greatly simplified. There are numerous periplasmic permeabilization methods using reverse micellar solution [5, 6, 11], organic solvents [1, 10], heat treatment [16], detergents [22], pulse electric field [20], osmotic shock [9, 18], EDTA [19] and chloroform [2], lysozyme/EDTA [7, 17]. There are some attempts for release of large scale. Although these methods work efficiently on a laboratory, they involve too many steps. Also, a potential difficulty with the use of large scale method is the presence of sucrose which can have a

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side effect on subsequent downstream processing. Sucrose increases the viscosity of the processing solution. An increase in viscosity reduces the efficiency of centrifugation or microfiltration. Thus, it is important to find a simple extraction method of recombinant enzyme from periplasmic space in *E.coli*.

Here we report the effect of mixture of detergent and lysozyme on release of penicillin G amidase from periplasmic in *E.coli*. Especially, kinds of detergent, concentration of detergent, reaction time, pH and temperature were investigated. This extracted enzyme was immobilized on porous silica resin without further purification. This immobilized penicillin G amidase was successfully applicable to produce 6-APA.

Materials and Methods

Materials

Molecular reagents were purchased from QIAGEN (Hilden, Germany), Invitrogen (San Diago, CA, U.S.A.), Gene clean Spin Kit (Bio 101). All other reagents was ordered from Sigma-Aldrich (U.S.A.), The employed main apparatus are shown as follows; thermal cycler(Gene Amp PCR system 2700; Applied Biosystems, U.S.A.), 50-L fermentor (KF-50L ; kobiotech, korea), sugar analyzer (YSI model 2700 STAT, Yellow Springs Instrument Inc., Ohio, U.S.A.) spectrophotometer (UV-265; Shimadzu, Japan), HPLC (Water, MA, U.S.A.)

Bacterial strains and plasmid

E.coli BL21 (DE3) and pET-24a (+) were obtained from Novagen (Madison, WI, U.S.A.). Expression plasmid pET-pga was used for the experimental procedure. The cloning method for expression of PGA was described in previous paper [14].

Fed batch culture condition

The pre-culture medium was LB containing kanamycin (50 μg/ml). Seed culture was prepared by inoculating 50 ml medium with a colony from fresh agar plate. Cells were incubated in a shaking incubator (250 rpm) during 7 hr at 37℃. The main medium for expression of PGA consisted of 10 g/l glucose, 5 g/l Yeast Extract, 4 g/l KH_2PO_4, 8 g/l $Na_2HPO_4{\cdot}12H_2O$, 5 g/l $MgSO_4{\cdot}7H_2O$, trace elements, 50 mg/l kanamycin, 20 g/l lactose and 0.2 g antifoaming agent. Fed batch fermentation was carried out 50 l stirred tank (20 l starting volume).

Cultures were inoculated with a 0.2%(v/v) seed culture. Air-saturation was maintained at 25% of dissolved oxygen concentration through increase the rpm, aeration rate up to 1.5 vvm, and some manual change of internal pressure. The substrate feeding strategy was glucose concentration control method based on pH-stat and glucose concentration in the culture broth was maintained below 0.1%. The main fermentation was started at 37℃ and decrease to 22℃. The temperature shift fermentation was performed as described previously [14].

Optimization of conditions for periplasmic PGA release

Cultured cells that treated with 2% $CaCl_2$ were harvested by centrifugation at 4,000 g. The cell pellets were resuspended with 100 mM phosphate buffer (pH 5.5~7.5), 100 mM Tris buffer (pH 8.5~9.5). Each buffer contains 0.1~1% detergents and 0.01~0.5% lysozyme. After samples were incubated 30 min at R.T. in shaking incubator (300 rpm), the cells were pelleted by centrifugation at 13,000x g for 30 min and the supernatants were collected as the periplasmic fraction. The precipitated cells were used for the cytoplasmic fraction. The cytoplasmic fraction was prepared by sonication and centrifugation (13,000x g).

PGA extraction in pilot scale

A 25-l culture was harvested in 5,000× g. The cell pellets from 25 l of fermentation broth was resuspended with 0.3% para-tertiaryoctylphenyl prooxyethylene, 0.1% lysozyme, 100 mM phosphate buffer (pH 7.5). The suspension was incubated for 30 min at 25℃. The fraction of PGA was recovered by diatomite filtration and removed particles by M/F. The extracted PGA fractions were desalted and concentrated with U.F. (M.W.C.O. 50 kDa).

Preparation of nanoporous silica particle

Nanoporous silica particles prepared were prepared by the procedure reported in previous paper [15]. 18% sodium silicate 15% H_2SO_4 were mixed through nozzle using pump. After formation of silica hydrogel, resulting mixture was washed with R/O water and condensed at 70℃, pH 9.0 for 60 hr. Washed silica hydrogel was dried at 120℃ and 8 hr. And dried silica hydrogel was maintained at 180℃ for 2 hr. under pressure (8~9 Kg/Cm^2). For hydro thermal treatment, nano silica particles were dried at 120℃ for 8 hr.

Treatment of butyl silane on nanoporous silica resin

The treatment of butyl silane was carried out as follows; butyl silane with 10%(w/w) were mixed with nanoporous silica resin at pH 7.5 and dried 130℃ for 5 hr.

Enzyme immobilization procedure

Butyl silanized nanopourou silica matrix with PGA solution (15 mg/ml, w/w: 1/1) and D.W. was added. Resulting mixture was stirred at 200 rpm, 30℃ for 15 hr. Immobilizes nano silica resin was harvested and washed 3 times with D.W. The sample obtained was dried at 35℃ for 5 hr. The yield of PGA immobilized to the resin was determined by the difference between initial and residual enzyme concentrations.

Analytical methods

Cell growth was determined by optical density measurements at 600 nm wave length using a spectrophotometer. Glucose concentration was monitored using glucose analyzer (YSI 2700 STAT, Yellow Springs Instrument Inc., Ohio, USA). The protein were analyzed by 12% SDS-PAGE and stained with Coomassie brilliant blue R 250. PGA activity was assayed by spectrophotometric method with 6-nitro-3-phenylacetamidibenzoic acid (NIPAB) and HPLC. Potassium penicillin G (PGK) hydrolysis produced 6-APA (6-aminopenicillanic acid).

Enzyme activity was calculated as follows;

$$\text{Activity (U/ml)} = \frac{\{\text{substrate conc. after 2 min reaction(mol)} - \text{substrate conc. after 10 min reaction (mol)}\}}{\text{amount of enzyme used (ml)} \times 8}$$

The products of enzyme reaction were analyzed by HPLC system built with a RP-HPLC C18 analytical column (Hypersil OSD, 5 um, 250 mm x 4.6 mm). A mobile phase consisted of 50 mM phosphate buffer (pH 6.0) with 16% ACN (acetonitrile) and was followed at 1 ml/min of flow rate. Absorbance at 225 nm of wave length was monitored by a U.V. detector.

Results

Optimization of conditions for periplasmic PGA release

Effects of various surfactants on PGA release

The release methods of PGA from periplasmic space in cells were performed a small scale from 100 ml culture broth. We tried to find the most efficient surfactant for PGA release from periplasmic space. The cell suspension was added with 0.1%, 1% various detergent and stirred at 30 min, 25℃. It is shown the effects of various kinds of surfactant on periplasmic PGA release in Fig. 1. After reaction, the reactants were centrifuged. The PGA activities of supernatant were analyzed. The enzyme activity extracted by anionic detergents such as SDS, ADS, was 30~37 U/ml. The yield of ADS extraction was higher than that of SDS extraction. Cationic detergent treatment (CETAB) was shown the activity of 25~30 U/ml. The yield of PGA release using non-ionic detergent, p-TOPPOE (Triton X-100) was 30~33 U/ml.

Treatment with mixture of surfactants and lysozyme

The cells were resuspended in phosphate buffer (pH 7.5) containing a different concentration of lysozyme (0.01%, 0.1%, 0.5%) and 1% surfactants. Those were incubated at 25 ℃, 300 rpm for 320 min, and harvested centrifugation to obtain the periplasmic fraction. Surfactant/lysozyme combination treatment increased the extraction yield of PGA from the periplasmic in comparison with the single surfactant treatment (Fig. 2). All cases were approximately almost two-fold higher (55~65 U/ml). Especially, non-ionic detergent, p-TOPPOE (Triton VX-100)/lysozyme treatment was recorded the best yield. Generally, the enzyme release was increased according to the lysozyme concentration increment.

Effect of pH

The effect of pH on the results of PGA release is shown in Fig. 3. The condition was 0.1% lysozyme, 1% detergent. In the case of SDS, ADS, CETAB treatment, it was obtained

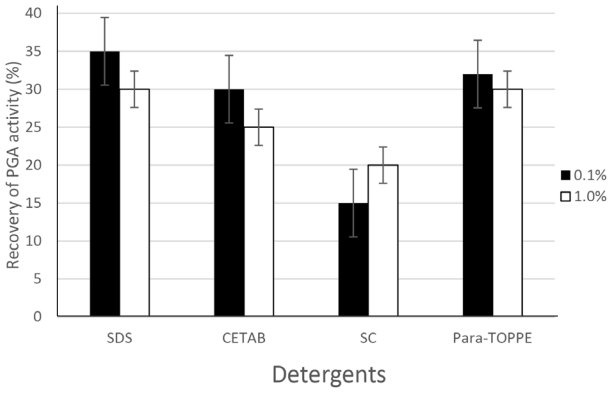

Fig. 1. Effect of detergent on the recovery of recombinant PGA from the periplasm of *E.coli*. SDS: sodium dodecyl sulfate, ADS: ammonium dodecyl sulfate, CETAB: cetyltrimethyl ammonium bromide, SC: sodium cholate, para-TOPPOE: para-tertiary otyl phenyl polyoxyethylene.

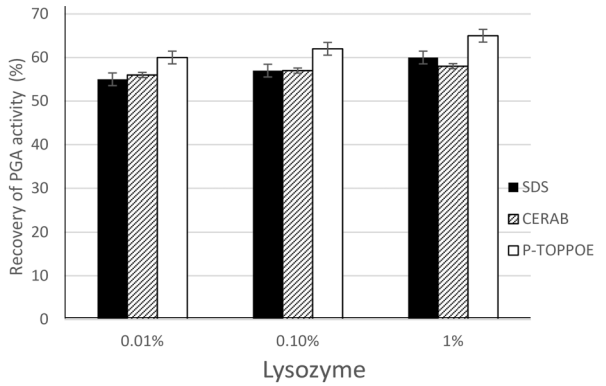

Fig. 2. Effect of lysozyme treatment on the release of recombinant PGA from *E.coli* at 1% detergent SDS: sodium dodecyl sulfate, ADS : ammonium dodecyl sulfate, CETAB : cetyltrimethyl ammonium bromide, para-TOPPOE : para-tertiary otyl phenyl polyoxyethylene.

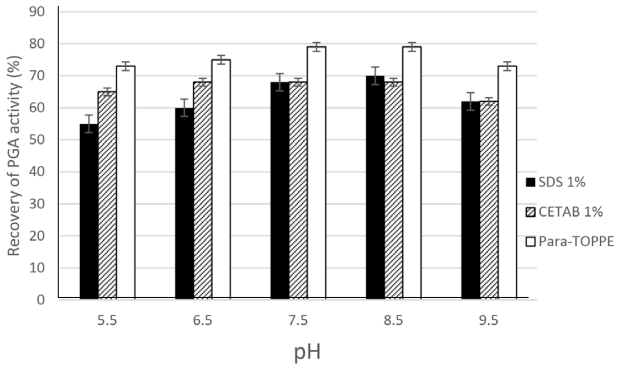

Fig. 3. Effect of pH on the release of periplasmic PGA from *E.coli*.

better yield of PGA release at 7.5, 8.5 than those at 5.5, 6.5, 9.5. However, Triton X-100 treatment was not an effect on enzyme release depending on pH but extracted greater levels of PGA from periplasm (>70 U/ml). The consistency of yield according to pH have a great advantage during the scale up the process.

Effect of temperature

All conditions except temperature were fixed (1% Triton X-100, 0.1% lysozyme, pH 7.5, 300 rpm, 30 min). The temperature range between 20℃ and 40℃ also was of no effect on the release of periplasmic PGA (Fig. 4.).

Effect of reaction time

The conditions except reaction time were the same (1% Triton X-100, 0.1% lysozyme, pH 7.5, 300 rpm, 25℃). The release of PGA was detected in accordance with the reaction time (5, 10, 15, 20, 25, 30 min). The activity of PGA was not increased after 25 min. These results indicate the reaction

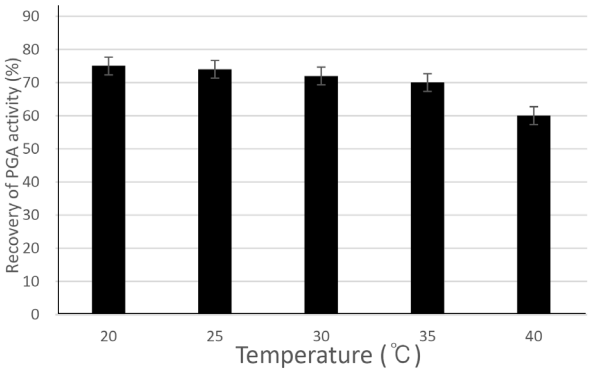

Fig. 4. Effect of temperature on the permeabilization of PGA from the periplasm of *E.coli* 1% p-TOPPOE, 0.1% lysozyme, pH 7.5.

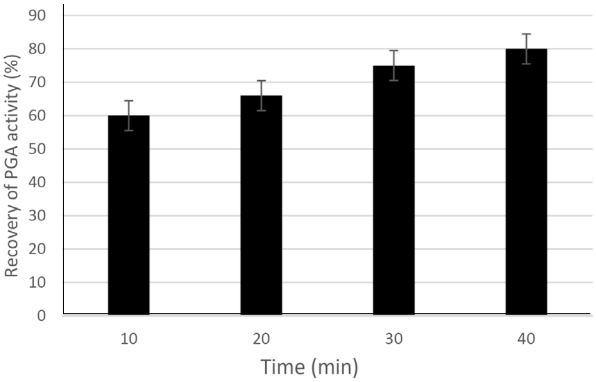

Fig. 5. Effect of reaction time on the recovery of PGA from the periplasm of *E.coli* Reaction conditions were fixed at 1% p-TOPPOE, 0.1% lysozyme, pH 7.5, 300 rpm.

time (30 min) is sufficient time for release of periplasmic PGA (Fig. 5).

Pilot scale release of PGA from periplasm

A 25-L *E.coli* BL21 (DE3) containing pET-pga fed-batch culture was pelleted after 28 hr of growth using the strategy of temperature shift fermentation. The culture broth contained activity of 17.2 U/ml. The 78.6% of the total cellular penicillin G amidase (total activity was 430 KU) was ex-

Table 1. The recovery of PGA from recombinant *E. coil* by treatment with combination ADS and lysozyme

Process step	Total Activity (KU)	Specific Activity (U/mg)	Step Yield (%)	Purification Factor
Fermentation	430	0.56	100	1
Diatomite	292	8.4	67.9	15
M/F	274	8.4	93.8	15
U/F	212	11	77.3	19.6

Overall step Yield was 49.2%.

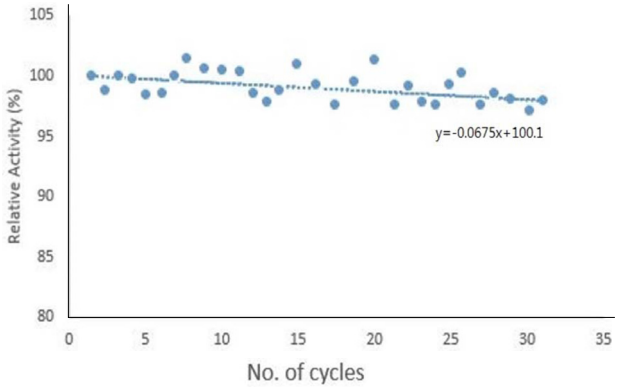

Fig. 6. Multi-cycle operation with the immobilized PGA.

tracted using the combined surfactant/lysozyme method. The step yield and specific activity in each process step are summarized in Table 1. It is shown the each step yield of diatomite filtration, membrane filtration (M/F), and ultrafiltration (U/F) was 69.7%, 93.8%, and 77.3%, respectively. Finally, total 212 KU of penicillin G amidase was recovered. At 25-L culture scale, the overall yield of extraction using the combined surfactant/ lysozyme method was 49.2% of the total cellular penicillin G amidase. The specific activity of extracted PGA was 11 U/mg in protein.

PGA immobilization

The effective size of pore diameter for PGA immobilization was 40-50 min. Total immobilization yield of PGA on the resin was 48.7%. The enzyme activity was 101 U/g (wet support).

Recycle operation with the immobilized PGA

The recycle stability is a very important point in commercial and practical application. Thirty cycles of reaction were operated using the immobilized PGA. As the numbers of cycle were increased to thirty, the enzyme activity did not decrease nearly (Fig. 6). The rate of decrease was negligible.

Discussion

E.coli cell envelope consists of outer membrane, periplasm (containing peptidoglycan) and cytoplasmic membrane. Recombinant penicillin G amidase was expressed in the periplasmic space [14]. The outer membrane is composed of charged lipopolysaccharide (LPS) molecules bound to each other by Ca or Mg. The polysaccaride part of the LPS molecules is oriented outward providing the cell surface hydropolycity, while the lipid portion is oriented inwards forming a bilayered structure with the phospholipids, present in the inner leaflet of the outer membrane. The inner cytoplasmic membrane is made up of bilayer of phospholipids with embedded proteins. Cationic surfactant are supposed to act on the LPS in outer membrane. The positively charged surfactant would bind strongly to the negatively charged lipid and phospholipids in the cell envelope and caused complete cell disruption depends on the conditions. As well, anionic detergents and some nonionic detergents, in the presence of ions like Mg, augment the membrane structure by immobilizing the LPS monolayer [13]. However, anionic detergents (such as SDS and sodium sarcosinate) in the presence of Mg, caused specific disruption of inner cell membrane [21].

Organic solvent (such as 2-butoxyethanol) treatment yielded significantly reduced concentration of other host cell proteins, lipopolysaccharide endotoxin and DNA in the recovered protein solution. However, there are some problems in the method. First, Allen *et. al*. [1] concluded the solvent concentration range of 30~50% w/w is necessary for release of periplasmic protein from Gram-negative bacteria. In this case, solvent concentration is required for phase separation between solvent and aqueous phase. Also, heating is not good for maintenance of activity of enzyme.

Protein release methods that increase the permeability of the outer cell wall for primary recovery but avoid rupturing the inner cell membrane, reduce contamination of the recovered product with other host cell components and simplified final purification.

Lysozyme is the usual enzyme which catalyzes the degradation of N-acetylmuramic acid-N-acetyl-glucosamine bond (NAM_NAG) in the rigid cell wall peptidoglycan [17]. In the Gram-negative bacteria, surfactants may be destabilize the outer membrane in mild condition and allow lysozyme to penetrate the peptidoglycan and hydrolyze the NAM-NAG bond of this layer.

The lysozyme in mixed with surfactant was effective for release of periplasmic penicillin G amidase from recombinant *E.coli*. It was applied the extraction method of protein from periplasmic space of recombinant *E.coli* to the immobilization of PGA on the nanoporous silica resin. The immobilized PGA was possible to use 30 times repeatedly. The experimental results described here suggest that the process for production of immobilized PGA may be useful in a process of biotransformation for other industrial purposes.

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References

1. Allen, J. R., Patkar, A. Y., Frank, T. C., Donate, F. A., Chiu, Y. C., Shields, J. E. and Gustafson, M. E. 2007. Use of glycol ethers for selective release of periplasmic proteins from Gram-negative bacteria. *Biotechnol. Prog.* **23**, 1163-1170.
2. Ames, G. F., Prody, C. and Kustu, S. 1984. Simple, rapid, and quantitative release Of periplasmic proteins by chloroform. *J. Bacteriol.* **160,** 1181-1183.
3. Baneyx, F., Ayling, A., Palumbo, T., Thomas, D. and Georgiou, G. 1991. Optimization of growth conditions for the production of proteolytically- sensitive proteins in the periplasmic space of *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **36,** 14-20.
4. Baneyx, F. and Mujacic, M. 2004. Recombinant protein folding and misfolding in *Escherichia coli*. *Nat. Biotechnol.* **22,** 1399-1408.
5. Bansal-Mutalik, R. and Gaikar, V. G. 2004. Mass transfer studied of cell permeabilization and recovery of alkaline phosphatase from *Escherichia coli* by reverse micellar solutions. *Biotech. Prog.* **20,** 1121-1127.
6. Bansal-Mutalik, R. and Gaikar, V. G. 2003. Purification and concentration of alkaline phosphatase by selective permeabilization of *Escherichia coli* using reverse micellar solutions. *Biotechnol. Prog.* **19,** 1713-1720.
7. French, C., Eli, K. M. and Ward, J. M. 1996. Development of a simple method for the recovery of recombinant proteins from the *Escherichia coli* periplasm. *Enzyme Microb. Technol.* **19,** 332-338.
8. Chandel, A. K., Rao, L. V., Narasu, M. L. and Singh, O. V. 2008. The realm of penicillin G acylase in beta-lactam antibiotics. *Enzyme Microb. Technol.* **42,** 326-330.
9. Chen, Y. C., Chen, L. A., Chen, S. J., Chang, M. C. and Chen, T. L. 2004. A modified osmotic shock for periplasmic release of a recombinant creatinase from *Escherichia coli*. *Biochem. Eng. J.* **19,** 211-215.
10. De Leon, A. B., Garcia, A. P., Barrba de la Rosa, F., Villasenor, A. Estrada and Lopez-Revilla R. 2003. Periplasmic penicillin G acylase activity in recombinant *Escherichia coli* cells permeabilized with organic solvents. *Process. Biochem.* **39,** 301-305.
11. Gaikar, V. G. and Kulkani, M. S. 2001. Selective reverse micellar extraction of penicillin acylase from *Escherichia coli*. *J. Chem. Tech. Biotech.* **76,** 729-736.
12. Jonasson, P., Nygren, P. A., Jornvall, H., Johansson, B. L., Wahren, J., Uhlen, M. and Stahl, S. 2000. Integrated bioprocess for production of human proinsulin C-peptide via heat release of an intracellular heptameric fusion protein. *J. Biotechnol.* **76,** 215-226.
13. Kim, J. K., Park, J. K. and Kim, H. K. 2004. Synthesis and characterization of nanoporous silica support for enzyme immobilization. *Colloids and surfaces A: Phsicochem. Eng. Aspects.* **241**, 113-117.
14. Lee, S. M. 2008. Fed-Batch culture of recombinant *E.coli* for the production of penicillin G amidase. *Kor. J. Microbiol. Biotechnol*. **36,** 314-319.
15. Nixdorff, B. 1978. Interaction of LPS with detergents and its possible role in the detergent resistant of the outer membrane of the Gram-negative bacteria. *Biochim. Biophys. Acta.* **510,** 87-98.
16. Pierce, J. J., Robinson, S. C., Ward, J. M., Keshavarz-Moore, E. and Dunnill, P. A. 2002. Comparison of the process issues in processing the same recombinant enzyme periplasmically in *Escherichia coli* and extracellularly in *Streptomyces lividans*. *J. Biotechnol.* **92,** 205-215.
17. Pierce, J. J., Turner, C., Keshavarz-Moore, E. and Dunnill, P. 1997. Factors determining more efficient large-scale release of a periplasmic enzyme from *E.coli* using lysozyme. *J. Biotechnol.* **58,** 1-11.
18. Rathoree, A. S., Bilbrey, R. E. and Steinmeyer, D. E. 2003. Optimization of an osmotic shock procedure for isolation of a protein product expressed in *E. coli*. *Biotechnol. Prog.* **19,** 1541-1546.
19. Ryan, W. and Parulekar, S. J. 1991. Recombinant protein excretion in *Escherichia coli* JM103[pUC8]: Effects of plasmid content, ethylenediaminetetraacetate, and phenyl ethyl alcohol on cell membrane permeability. *Biotechnol. Bioeng.* **37,** 430-444.
20. Shiina, S., Ohshima, T. and Sato, M. 2004. Extracellular release of recombinant alpha-amylase from *Escherichia coli* using pulsed electric field. *Biotechnol. Prog.* **20,** 1528-1533.
21. Woldringgh, C. L. 1970. Lysis of the cell membrane of *Escherichia coli* K12 by ionic detergents. *Biochim. Biophys. Acta* **224,** 288-290.
22. Zhao, F. and Yu, J. 2001. L-asparaginase release from *Escherichia coli* cells with K_2HPO_4 and Triton X100. *Biotechnol. Prog.* **17,** 490-494.

초록 : 대장균의 periplasm으로부터 재조합 PGA 단백질의 효율적이고 간단한 방출 방법

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세제에 의하여 대장균의 periplasm에서 penicillin G amidase (PGA)를 방출하는 방법을 연구하였다. 결과적으로 세제와 lysozyme의 혼합 작용이 효과적인 것으로 나타났다. 세포 투과성의 최적 조건을 알아보기 위하여 세제의 종류, 농도, pH, 반응 시간, 온도 등의 영향을 살펴보았다. 그리하여 대장균에서 재조합 PGA를 periplasm에서 방출하는 모델을 만들 수 있었고 방출된 PGA를 농축할 수 있었다. 실리카 구슬을 이용한 고정화 시스템으로 PGA 용액을 농축할 수 있었으며, 더 이상의 정제 과정 없이 순수하게 추출 할 수 있었다. 고정화된 PGA는 penicillin G 생성의 원료인 6-APA를 생산하는데 사용할 수 있었다. 이 방법은 대장균으로부터 재조합 단백질을 추출하는 간단한 방법이며 고정화 PGA를 이용하여 β-lactam 항생물질의 산업적 생산 이용될 수 있을 것으로 사료된다.