Journal of Science and Technology ***54*** *(4A) (2016) 123-131*

EXPRESSION OF GLUTARYL7-AMINOCEPHALOSPORANIC ACID ACYLASE IN *ESCHERICHIA COLI* BL21(DE3) AND IMMOBILIZATION OF RECOMBINANT ENZYME ON NANOPOROUS MATERIALS

Vu Thi Hanh Nguyen, Pham Thanh Huyen, Le Gia Hy, Phi Quyet Tien*

Institute of Biotechnology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

*Email: *tienpq@ibt.ac.vn*

Received: 15 August 2016; Accepted for publication: 5 October 2016

ABSTRACT

The synthesis of 7-ACA from cephalosporin C (CPC) by a two-step bioconversion using D-amino acid oxidase (DAAO) and glutaryl 7-ACA acylase (GLA) has been effectively and largely applied in pharmaceutical industry. In this study, the gene *gla* coding for 720-amino acid GLA from plasmid pUC57::*gla* was analyzed and successfully inserted into vector pET22b(+) to form expression vector pET22b(+)::*gla*. The newly constructed expression vector pET22b(+)::*gla* was cloned and then transformed into *Escherichia coli* BL21(DE3) to generate recombinant strain *E. coli* BL21(DE3)[pET22b(+)::*gla*]. The suitable conditions for expression of *gla* gene were in LB medium at 30 ^{o}C and induced by 0.4 mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG) for 3 hours. Under the chosen culturing parameters, expression of *gla* gene by *E. coli* BL21(DE3)/[pET22b(+)::*gla*] resulted in a recombinant GLA (rGLA) with molecular weight of 83 kDa and catalytic activity of 2.7 U/mg of total protein. Experimental research on immobilization of rGLA onto ten nanoporous materials were showed that, SBA-15 was the best one for immobilization of rGLA, reaching activity of immobilized enzyme of 22.2 U/g matrix. Furthermore, optimal conditions of procedure for immobilizing rGLA on nanomaterials (SBA-15) were determined as follows: temperature is 25 °C, pH7.0 and immobilization time –60 minutes. Therefore the results reported in this study revealed the successfully heterologous expression of GLA in recombinant *E. coli* and potential immobilization of enzyme on inorganic nano-materials.

Keywords: 7-ACA, Cephalosporins, *E. coli*BL21(DE3)/[pET22b(+)::gla], enzyme immobilization, GLA, SBA-15.

1. INTRODUCTION

7-Aminocephalosporanic acid (7-ACA) is a very useful intermediate in the production of medically important semisynthetic cephalosporins such as cephalaglycin and cephalothin [1]. 7-ACA has been produced industrially by the deacylation of cephalosporin C (CPC) by chemical methods. Because of the environmental and safety concerns, enzymatic conversion of CPC has

long been explored as a substitute for chemical methods [2]. The enzymatic conversion of CPC to 7-ACA was carried out by two methods [3]: (i) one-step process using cephalosporin C acylase which effectively uses CPC as substrate; however, the catalytic efficiency of these enzymes is weak thus their application is limited; (ii) two-step process comprising the conversion of CPC into GL 7-ACA, using DAAO, and its subsequent hydrolysis to 7-ACA by a GLA which have been widely applied in 7-ACA industrial production [4]. The genes coding for GLA from several *Pseudomonas* species have been expressed in *E. coli* and the enzymes were biochemically analyzed. The enzymes were found to be fully active even in a foreign host, as *E. coli* [5]. In *E. coli*, GLA was secreted into the periplasmic space [6]. In the present study, we described the cloning, nucleotide sequence and high-expression of GLA in *E. coli* BL21(DE3) and immobilization of recombinant enzyme on nanoporous materials.

2. MATERIALS AND METHODS

2.1. Materials

The strains *Escherichia coli* XL1-blue and *E. coli* BL21(DE3) (Stratagene, USA). Plasmid pUC57::*gla* harboring *gla* gene was kindly provided by Fermentation Technology Laboratory, Institute of Biotechnology (IBT), VAST. Plasmid pET22b(+), *Eco*RI and *Sac*I, ligation enzyme T4 ligase, PureLink™ - DNA purification, PureLink™ - Plasmid Extraction kits (Invitrogen, USA). Chemicals were purchased from Mecrk (Germany), Invitrogen and Fermentas (USA) and other providers. Nanoporous materials SBA-15, SBA-16, MCF and MCM were supplied by Departement of Surface Sciences and Catalysis, Institute of Chemistry, VAST.

2.2. Methods

2.2.1. Analysis of gla gene sequence in pUC57::*gla*: The sequence of *gla* gene in pUC57::*gla* was sequenced by using primers M13-F and M13-R in machine ABI PRISM®3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by National Key Laboratory of Gene Technology, IBT, VAST. The sequence was analyzed by DNA-Star Software (Madison, WI, USA) and aligned by BLAST tool on NCBI (www.ncbi.nlm.nih.gov).

2.2.2. DNA transformation in E. coli by heat-shocked method: The heat-shock transformation of DNA into *E. coli* cells was done by method described by Sambrook*et al*. [7].

2.2.3. Construction of expression plasmid pET22b(+)::gla: The plasmids pUC57::*gla* and pET22b(+) were dually cut by *Eco*RI and *Sac*I, then gene products were purified by PureLink™–DNA Purification (Invitrogen, USA) and ligated by T4 ligase (Invitrogen, USA). The ligation mixture of *gla*gene and pET22b(+) were transformed into competent *E. coli* XL1-blue by heat-shock method and spread onto the agar-LB/amp medium. Growing transformants were then cultured in broth LB/amp and plasmid was extracted by PureLink™–Plasmid Extraction Kit (Invitrogen, USA). The presence of *gla*gene in newly constructed expression plasmid pET22b(+)::*gla* was checked by PCR using specific primers and cut by both *Eco*RI and *Sac*I. The recombinant strain *E. coli* BL21(DE3)/[pET22b(+)::*gla]* was then generated by transforming vector pET22b(+)::*gla* into *E. coli* BL21(DE3). The recombinant *E. coli* BL21(DE3)/[pET22b(+)::*gla]* was used for expression of rGLA in further experiments.

2.2.4. Expression of rGLA in E. coli BL21(DE3)/[pET22b(+)::*gla]:* The strain *E. coli* BL21(DE3)/[pET22b(+)::*gla]* was cultured in broth LB/amp overnight at 37 ^{o}C on rotary shaker of 220 round per munite (rpm) to prepare pre-culture. An amount of 0.5 ml of pre-culture was transferred into 5 ml of fresh LB/amp medium and cultivated at 37 ^{o}C with shaking of 220 rpm until OD_{600nm} of culture reached up 0.6 unit. Then, the expression of rGLA was induced by isopropylthiogalactoside (IPTG) at final concentrations ranging from 0.05 - 1.0 mM. The control sample was done without induction with IPTG. To choose the harvested time, the strain *E. coli* BL21(DE3) was induced and grown at selected IPTG concentration and temperature. The suitable harvested time was selected by choosing the best rGLA activity expressed among samples collected at 1- 6 hours after induction.

*2.2.5. Assay of rGLAactivity:*Preparation of rGLA crude extract as follows: The *E. coli* BL21(DE3)/[pET22b(+)::*gla*] cells were harvested by centrifugation at 6.000 rpm for 15 min at 4^{o}C, washed and resuspended in 100 mM Tris HCl-100 buffer pH 8 containing 0.1 % Triton X-100 (v/v), sonicated for 2 min and supernatant of cell lysate were used as crude extract of rGLA [7]. rGLA activity was determined by the method described by Nohair *et al.* [8].

2.2.6. Immobilization of rGLA on nanomaterials: The procedure for immobilization of rGLA on nanoporous materials was performed as follows method Nohair *et al*.[8].

2.2.7. Calculation of immobilization yield: Efficacy of immobilization was calculated as the ratio of the amount of protein bound on the carrier to the initial amount of protein. Yield was expressed in a percentage. The amount of protein was determined by the Bradford's method [7].

3. RESULTS AND DISCUSSION

3.1. Sequence of *gla* gene in plasmid pUC57::*gla*

To analyze the sequence of *gla* gene coding for GLA, the gene inserted in plasmid pUC57::*gla* was sequenced. The sequencing data showed an ORF of 2,160 bp coding for 720-amino acid protein. The ORF shows high homology (about 98 – 99 %) with *gla* genes from *Pseudomonas* sp. deposited on GenBank (NCBI). Examination of recognition site by restriction enzymes found that there were no recognition sites for *Eco*RI and *Sac*I. Therefore, two restriction sites *Eco*RI and *Sac*I could be used for insertion of *gla* gene into vector pET22b(+) at MCSs positions.

3.2. Construction of expression vector pET22b(+) harboring *gla* gene

Based on mentioned data, gene *gla* was cut from pUC57::*gla* by both enzymes *Eco*RI and *Sac*I, purified and then ligated into pET22b(+) pre-treated with both *Eco*RI and *Sac*I. The ligated mixture was then transformed into *E. coli* XL1-blue and recombinant plasmids in ten colonies of *E. coli* transformants were subsequently extracted.

In comparison with length of native plasmid pET22b(+), all 10 extracted plasmids showed the length larger of than that of pET22b(+) without DNA insertion (Lane C). Therefore, the obtained result demonstrated that all 10 plasmids in clones could be successfully constructed by ligating *gla* gene into vector pET22b(+) (Fig. 1). To confirm the correct insertion of *gla* gene into pET22b(+), recombinant plasmids from three clones 5, 6 and 10 were randomly selected and treated with *Eco*RI, *Sac*I and the presence of *gla* gene was checked by PCR method using

the pair of primers GLA-F and GLA-R (Fig. 2). Random treatment of three plasmids from clones 5, 6, 10 with *Eco*RI or *Sac*I (Fig. 2A) showed single DNA band of approximately 7.7 kb, corresponding to total side of *gla* gene (2174 bp) and plasmid pET22b(+) (5493 bp). When three plasmids (lanes 5, 6, 10) were linearized by either *Eco*RI or *Sac*I, the plasmid size is about 7.7 kb which include gene *gla* of 2.2 kb and plasmid pET22b(+) of about 5.5 kb, respectively (Fig. 2A and 2B). The comfirmation of the presence of *gla* gene in three plasmids by PCR using specific primers GLA-F and GLA-R (Fig. 2B) showed single bands of 2.2 kb on lanes 7, 8, 9. Besides, the PCR amplification using pUC57::*gla* as DNA template also exhibited the single DNA band of 2.2 kb (lane C+) in size (Fig. 2B).

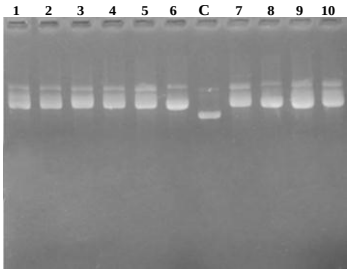

Figure 1. Analysis of recombinant plasmid forming by ligation of *gla* gene and vector pET22b(+) on agarose gel. **Lane C**: Vector pET22b(+). **Lane 1** to **10**: Recombinant plasmids randomly extracted from *E. coli* XL1-blue.

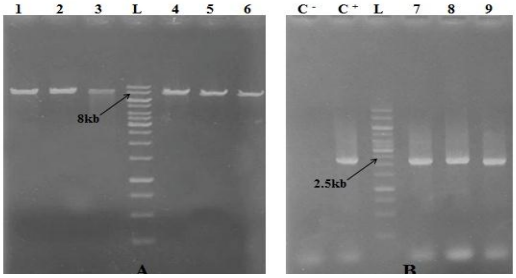

Figure 2. Agarose electrophoretic analysis of plasmids from three clones 5, 6, 10 cut by *Eco*RI and *Sac*I (A) and Amplification of *gla* gene by PCR using plasmids as DNA templates (B). **Lane L**: Ladder 1kb; **Lane 1, 2, 3**: Plasmids from clones 5, 6, 10 cut by *Sac*I; **Lane 4, 5, 6**: Plasmids from clones 5, 6, 10 cut by *Eco*RI, respectively; **Lane 7, 8, 9**: PCR products using plasmid from clones 5, 6, 10; **Lane C$^-$**: Negative control (H_2O as template); **Lane C+:** Positive control using pUC57::*gla* as the DNA template.

Recombinant plasmid from the clone 5 was choosen to transfer into expression host *E. coli* BL21(DE3) to form recombinant *E. coli* BL21(DE3)[pET22b(+)::*gla*]. Four colonies extracted from *E. coli* BL21(DE3)/[pET22b(+)::*gla*] tranformants on agar LB/amp were then cultivated in broth LB/amp for further plasmid extraction. Four extracted plasmids were separated on agarose electrophoresis to compare the sizes with that of native plasmid pET22b(+) (data not shown). The resulting plasmids pET22b(+)::*gla* extracted from *E. coli* BL21(DE3)/[pET22b(+)::*gla*] were cut with each of *Eco*RI, *Sac*I and used as the DNA template for amplification of *gla* gene by PCR (data not shown). From the obtained results, it can be concluded that recombinant vector pET22b(+)::*gla*was successfully transformed into expression host *E. coli* BL21(DE3) to form the recombinant *E. coli* BL21(DE3)/[pET22b(+)::*gla*]. The strain *E. coli* BL21(DE3)/[pET22b(+)::*gla*] was consequently used to express rGLA in next experiments.

3.3. The conditions for expression of rGLA by *E. coli* BL21(DE3)/[pET22b(+)::*gla*]

3.3.1. IPTG concentration

The crude extract of rGLA from cultures of recombinant *E. coli* BL21 induced by different IPTG concentrations were analyzed (Fig. 3, Fig. 4). The intensity of the 83 kDa band, indicating the expression level of recombinant rGLA, from cultures induced by IPTG ranging from 0.05 - 1.0 mM showed differences in which band from 0.4 mM IPTG induction revealed the highest level (Fig. 3). In the meanwhile, the examination of rGLA activities demonstrated that rGLA

was produced at high levels in range of 0.2 to 0.6 mM IPTG induction and decreased with higher IPTG concentration of 0.6 mM. Among those, the highest rGLA activity was about 2.7 U/mg protein when the culture of *E. coli*BL21(DE3)/[pET22b(+)::*gla*] was induced by 0.4 mM IPTG. Thus, SDS-PAGE analysis of rGLA showed two bands of 54 and 16 kDa corresponding to α and β-subunit [9]. However, no distinct separation of two subunits was observed in electrophoresis results of rGLA samples induced in this study although crude extract still showed enzyme activity.

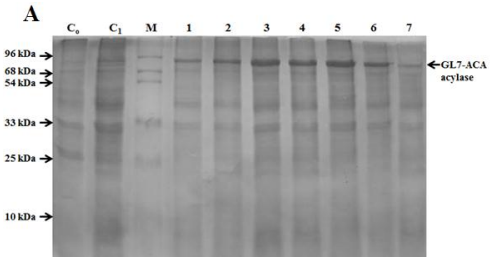

Figure 3. SDS-PAGE analysis of protein crude extracts (A) and rGLA activity tests from from culture of *E. coli*BL21(DE3)/[pET22b(+)::*gla*] induced by different concentration of IPTG.

Lane M: Protein marker
Lane C_0: Negative control using protein crude extract of *E. coli* BL21(DE3)
Lane C_1: Negative control using protein crude extract *of E. coli* BL21(DE3)/[pET22b(+)::*gla]* without IPTG induction
Lane 1, 2, 3, 4, 5, 6: *E. coli* BL21(DE3)/[pET22b(+)::*gla]* induced by IPTG concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM , respectively.

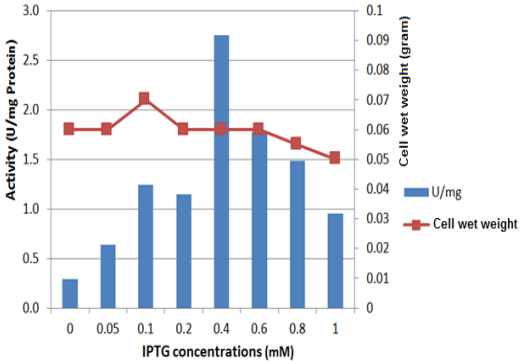

Figure 4. Effect of concentration of IPTG on the rGLA activity of *E. coli* BL21(DE3)/[pET22b(+)::*gla*]

3.3.2. Incubation temperature

The expression of rGLA was demonstrated to depend on the growth temperature [10]. To investigate the effect of growth temperature on rGLA productivity, the culture of recombinant *E. coli* BL21 was induced with 0.4 mM of IPTG at the range of temperatures of 25 ^oC, 30 ^oC, 32 ^oC and 37 ^oC for harvest times of 3 hours. As shown in Table 1, the yield of rGLA was dependent on the growth temperature with the highest activity (reaching up 109.5 %) compared with that expressed at 25 ^oC. In the frame of this study, the harvest times for expression of rGLA at various temperatures were selected according to previous literatures reported. The highest activity of rGLA was reached at 30 ^oC.

3.3.3. Harvested time for expression of rGLA

To investigate the effect of harvested time on rGLA productivity, the culture of the strain *E. coli*BL21(DE3)/[pET22b(+)::*gla*] were induced by 0.4 mM of IPTG, incubated at 30^oC and shaking 200 rpm. Activities of rGLA were compared with that harvested after 4 hours of induction at 30 °C (calculated as 100 %) (Fig. 5). rGLA activity was increased strongly after 1, 2 and 3 hours of induction at 30 ^oC and reached the highest level of 102 % after 3 - 4 hours. Moreover, *E. coli* BL21(DE3)/[pET22b(+)::*gla*] harvested time after 5 and 6 hours were slightly decreased, revealing that prolonged incubation time did not increase the rGLA productivity. The

suitable time for rGLA synthesis in *E. coli* BL21(DE3)/[pET22b(+)::*gla*] was 3 hours after induction.

Table 1. Relative activity of rGLA in cultures expressed at different temperatures.

Temperatura (°C)	Enzyme Activity (%)**	Cell wet weight (g)
C*	27.4	0.042
25	100	0.037
30	109.5	0.061
32	96.5	0.072
37	73.7	0.055

C*: Control *E. coli* BL21(DE3)/ [pET22b(+)::*gla]* without IPTG growing at 37°C and 2 hours
(%)**: The rate of samples enzyme activity compared

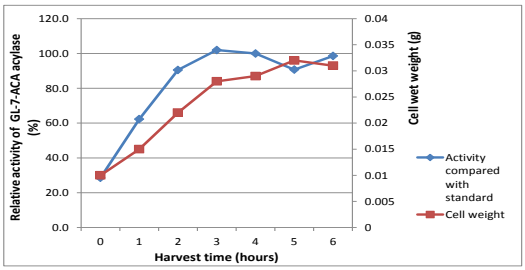

Figure 5. Effects of harvest time on the rGLA productivity of *E. coli* BL21(DE3)/ [pET22b(+)::*gla]*.

3.4. Suitable nanomaterials for immobilization of rGLA

Crude extract of rGLA was used directly for immobilization onto nanomaterials. Initial input parameters of rGLAactivity and protein concentration were fixed at start values of 2.7 U/mg protein and 4.02 mg protein/ml, respectively. Results of examination for immobilization of rGLA on ten kinds of nanoporous materials (SBA-15, SBA-15-VTES, SBA-15-PTMS, SBA-16-M4, SBA-16-S5-APTES, MCF1, MCF2, MCF6-APTES MCM41 and MCM41-APTS) were shown in Table 2. On the basis of data in Table 2, there were significant differences in the yields of rGLA immobilized on materials among groups of SBA-15, SBA-16, MCF and MCM. The activities of immobilized GLA were high when using supporting materials SBA-15, reaching acylase activities 22.2 U/g material.

Table 2. Summary of the activity of rGLA immobilized on different nanomaterials.

No	Nanoporous material	Efficacy of immobilization*(%)	Specific activity** (U/mg protein)	Enzymatic activity*** (U/g material)
1	SBA-15	27.6	0.56	**22.2**
2	SBA-15-VTES	10.4	1.4	14.1
3	SBA-15-PTMS	5.9	0.94	7.8
4	SBA-16-M4	**36.1**	0.28	14.6
5	SBA-16-S5-APTES	5.56	**2.1**	15.6
6	MCF1	**29.7**	0.38	14.4
7	MCF2	8.7	1.0	12.0
8	MCF6. APTES	2.7	0.92	3.5
9	MCM41	1.8	2.2	5.2
10	MCM 41-APTS	15.2	1.0	**21.4**

Note: VTES - Vinyl triethoxysilane; PTMS – Phenyl trimethoxysilane; APTES – amino propyl triethoxysilane; APTS - amino propyl triethoxysilane. ***** % protein immobilized after immobilization; ****** Enzymatic activity per 1mg of immobilized protein; ******* enzymatic activity per 1 g dry support materials

3.5. Appropriate conditions for immobilization of rGLA on SBA-15

Enzyme immobilized process is influenced by several factors such as temperature, time and pH of buffer solution [1]. The most suitable temperature for immobilization of rGLA on SBA-15 capillary is 25 ^{o}C (Fig. 6). At this temperature, enzyme activity and protein immobilized performance were the highest, reach to 23.2 U/g and 31.2 % protein. Therefore, choosing the optimal temperature for rGLA immobilization on SBA-15 is 25 $^{\circ}$C for next studies. At pH alkaline pH 9 - 10, the ability of immobilized rGLA on SBA-15 was not high, only 13.3 and 5.6 U/g, but optimum pH is pH 7.0 (reach to 31.2 U/g and 49.9 % protein) (Fig. 7). Meanwhile, the team of Park and Lee used silica gel or resins like amino polysiloxane, ethylene diaminepolysiloxane for GLA immobilization in phosphate buffer pH 8.0 [1, 11].

Immobilization time ofrGLA presented in Figure 8 showed that the immobilized enzyme activity was highest after 60 minutes reach to 36.6 U/g material and protein immobilized efficiency 39.5 %. If we continue to extend the time, the immobilized enzyme activity declined sharply, only 7.4 U/g after 330 minutes. The results of another study showed that the GLA immobilized on silica gel (180 minutes) or organic resin (240 minutes), it takes more time than this enzyme immobilized on SBA-15 [1, 11].

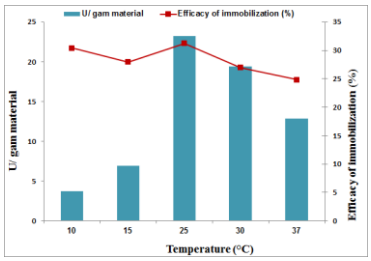

*Figure 6.*Effect of temperature for GLA immobilization on SBA-15.

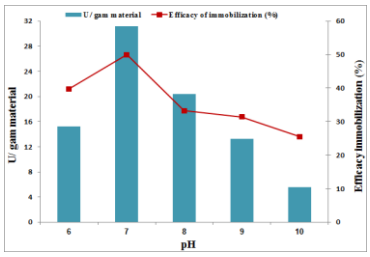

*Figure 7.*Effect of pH for GLA immobilization on SBA-15.

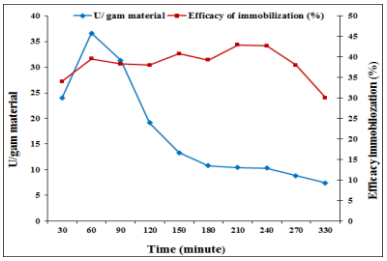

*Figure 8.*Effect of immobilization time to GLA on SBA-15.

4. CONCLUSION

By expression of *gla* gene in *E. coli* BL21(DE3), the recombinant GLA was successfully synthesized in LB medium at 30 ^{o}C and induced by 0.4 mM of IPTG for 3 hours. Biochemical analysis showed that the recombinant enzyme has molecular weight of about 83 kDa and specific activity of 2.7 U/mg of protein. Among of nanoporous materials for immobilization examination of rGLA, SBA-15 was the most appropriate material. The suitable conditions of procedure for immobilizing rGLA on SBA-15 were determined as follows: temperature is 25 °C, pH - 7.0 and immobilization time -60 minutes (reaching the highest activity was 36.6 U/g material).

Acknowledgements. This research was supported by the National Foundation for Science and Technology Development, Vietnam (NAFOSTED) under the project code 106.03-2011.07.

REFERENCES

1. Park S. W., Lee J., Hong S. I. and Kim S. W. - Enhancement of stability of GL 7-ACA acylase immobilized on silica gel modified by epoxide silanization, Proc. Biochem**. 39** (2003) 359-366.

2. Lee Y. H., Chang T. S., Liu H. J., and Chu W. S. - An acidic glutaryl-7-aminocephalosporanic acid acylase from *Pseudomonas nitroreducens*, Biotechnol. Appl. Biochem. **28** (1998) 113-118.
3. Sonawane V. C. - Enzymatic modifications of cephalosporins by cephalosporin acylase and other enzymes, Crit Rev Biotechnol **26** (2006) 95-120.
4. Volonte F., Marinelli F., Gastaldo L. *et al.* - Optimization of glutaryl-7-aminocephalosporanic acid acylase expression in *E. coli*, Protein Expr Purif. **61** (2008) 131-137.
5. Lee Y. S., Park S. S. - Two-step autocatalytic processing of the glutaryl 7-aminocephalosporanic acid acylase from *Pseudomonas* sp. strain GK16, J. Bacteriol.**180** (1998) 4576-4582.
6. Matsuda A., Komatsu K. I. - Molecular cloning and structure of the gene for 7 beta-(4-carboxybutanamido) cephalosporanicacidacylase from a *Pseudomonas* strain, J. Bacteriol. **163** (1985) 1222-1228.
7. Sambrook J., Russell D. W., Sambrook J. - The condensed protocols from Molecular cloning: a laboratory manual, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press, 2006, pp. 187.
8. Nohair B., Thao P. T. H., Nguyen V. T. H., Tien P. Q., Phuong T. D., and Kaliaguine S. - Hybrid periodic mesoporousorganosilicas(PMO-SBA-16): A support for immobilization of d-Amino acid oxidase and glutaryl-7-amino cephalosporanicacid acylaseenzymes, The Journal of Physical Chemistry *C***116** (2012) 10904-10912.
9. Kim D. W., Yoon K. H. - Cloning and high expression of glutaryl 7-aminocephalosporanic acid acylase gene from *Pseudomonas diminuta*, Kluwer Academic PublishersBiotechnology Letters **23** (2001) 1067–1071.
10. Khang Y.H., Yoo B.H. - Isolation and characterization of a novel soil strain, *Pseudomonas cepacia* BY21, with glutaryl-7-aminocephalosporanic acid acylase activity, Kluwer Academic Publishers **22** (2000) 317–20.
11. Lee S. K., Park S. W., Kim Y. I., Chung K. H., Hong S. I., and Kim S. W. - Immobilization of GL-7-ACA acylase for the Production of 7-ACA, Chem. Eng. **19** (2001) 161-166.

TÓM TẮT

NGHIÊN CỨU BIỂU HIỆN GENE GLUTARYL-7-AMINOCEPHALOSPORANIC ACID ACYLASE TRONG CHỦNG *ESCHERICHIA COLI* BL21(DE3) VÀ CỐ ĐỊNH ENZYME TÁI TỔ HỢP TRÊN VẬT LIỆU MAO QUẢN NANO

Vũ Thị Hạnh Nguyên, Phạm Thanh Huyền, Lê Gia Hy, Phí Quyết Tiến*

Viện Công nghệ sinh học, Viện Hàn lâm KHCNVN, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội

*Email: *tienpq@ibt.ac.vn*

Quá trình sản xuất của 7-ACA từ cephalosporin C tự nhiên thông qua chuyển hóa sinh học gồm 2 bước sử dụng D-amino acid oxidase (DAAO) và glutaryl-7-ACA (GL-7-ACA) acylase

(GLA) đã được ứng dụng hiệu quả và rộng rãi trong công nghiệp dược phẩm. Trong nghiên cứu này, gene gla mã hóa cho trình tự 720 amino-acid của GLA từ plasmid pUC57::*gla* đã được phân tích và chèn vào vector pET22b(+) tạo vector biểu hiện pET22b(+)::*gla*. Điều kiện thích hợp cho sự biểu hiện của rGLA là trong môi trường LB ở 30 °C và cảm ứng bởi 0,4 mM IPTG trong 3,0 giờ. Với điều kiện lên men đã chọn, sự biểu hiện của gene gla bởi *E. coli*BL21(DE3)/[pET22b(+)::*gla*] tạo ra GLA tái tổ hợp với khối lượng phân tử khoảng 83 kDa và hoạt tính enzyme đạt 2,7 U/mg protein tổng số. Nghiên cứu quá trình cố định rGLA trên 10 loại vật liệu mao quản nano cho thấy, SBA-15 là tốt nhất để cố định rGLA, hoạt tính enzym cố định đạt 22,2 U/g vật liệu. Hơn nữa, đã nghiên cứu điều kiện tối ưu để cố định rGLA từ *E. coli* tái tổi hợp trên vật liệu mao quản nano: nhiệt độ 25 °C, pH 7,0 và thời gian cố định là 60 phút. Kết quả trong nghiên cứu này cho thấy biểu sự hiện GLA trong *E. coli* đã thành công và tiềm năng cố định của enzyme nên các vật liệu nano.

Từ khóa: 7-ACA, Cephalosporins, *E. coli*BL21(DE3)/[pET22b(+)::gla], enzyme cố định, GL-7-ACA acylase, SBA-15.