Hue University Journal of Science: Natural Science
Vol. 129, No. 1D, 61–65, 2020

pISSN 1859-1388
eISSN 2615-9678

IMPROVED EXPRESSION OF CATECHOL 1,2-DIOXYGENASE GENE FROM *BURKHOLDERIA CEPACIA* IN *ESCHERICHIA COLI*

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(*Received: 22 August 2020; Accepted: 24 August 2020*)

Abstract. Catechol 1,2-dioxygenase (CAT1) is a key enzyme for the *ortho*-cleavage pathway involved in the degradation of dibenzofuran, a dioxin derivative, which is a highly toxic environmental pollutant. The present study aims to investigate appropriate culture conditions for enhancing the expression of the *cat1* gene encoding CAT1 enzyme from *Burkholderia cepacia* DF4 in *Escherichia coli* M15. The optimized culture conditions for gene expression are cell density at the time of induction, shaking speed, induction temperature, induction time, and inducer concentration. The highest level for CAT1 was obtained at the IPTG concentration of 1.2 mM, 10 hours after induction at 35 °C, shaking speed 200 rpm with cell density at OD_{600} 0.7.

Keywords: *Escherichia coli* M15, CAT1, gene expression, dibenzofuran

1 Introduction

Polychlorinated aromatic compounds with 2–3 aromatic rings and 2 to 8 chlorine atoms are extremely hazardous substances. These substances are collectively known as dioxins [1]. Dioxins and their derivatives are groups of hundreds of toxic and persistent compounds in the environment, mainly consisting of three groups of compounds: polychlorinated dibenzo-*p*-dioxin, polychlorinated dibenzofuran, and polychlorinated biphenyl [2].

Dibenzofuran is a polycyclic aromatic compound, an industrial chemical, used as a pesticide, PVC production, and bleaching industry [3, 4]. Dibenzofuran derivatives have caused serious environmental problems that posed a major challenge to human health [5].

The biological methods using specific microorganisms capable of dioxin transformation and degradation have become an interesting alternative to physicochemical methods in environmental improvement. A large number of dioxin and dioxin-like compounds degrading microorganisms have been isolated and characterized [6].

Environmental aromatic pollutants have been reported to be biodegraded by a variety of microorganisms that contain various dioxygenases capable of cleaving aromatic compounds. Several microorganisms use a catabolic pathway for the degradation of these compounds called β-ketoadipate pathway. Catechol 1,2-dioxygenase is the key enzyme in the β-ketoadipate pathway, catalyzing the cleavage of the aromatic ring of catechol to *cis*,*cis*-muconic acid with the incorporation of two atoms of molecular oxygen into the substrate [7].

Schlüter et al. reported a phenol-degrading yeast *Trichosporon mucoides* capable of degrading dibenzofuran, diphenyl ether, and biphenyl by secreting ring cleavage enzyme. Although CAT1 from this yeast strain is not capable of cleavage the aromatic ring system of 3,4-dihydroxyphenyl, it might catalyze the *ortho*-cleavage of dihydroxylated monoaromatic compounds [8]. The CAT1 isolated from *Rhodococcus ruber* OA1 is capable of degrading catechol, an intermediate metabolite in salicylic acid biodegradation. So far, *cat1* gene has been isolated from different microorganisms such as *Pseudomonas putida* N6, *Rhodococcus opacus* 1CP, *Geobacillus* sp. G27, *Sphingomonas xenophaga* QYY, and *Acinetobacter* sp. DS002, cloned and expressed in *E. coli* [9].

The use of catechol dioxygenase for bioremediation is relatively less researched although it has a potential for cleaning the wastewater contaminated with phenol, benzoate, fluorocatechol, bromocatechol, cholorocathechol, methilcathechol, herbicides (diuron), polychlorinated biphenyls, chloroethanes, and others. Therefore, the production of large amounts of catechol dioxygenase in the laboratory is absolutely necessary to supply a cheap recombinant enzyme for commercial use and environmental treatment [10]. In this study, the production of recombinant enzyme CAT1 is enhanced in *E. coli* cells by optimizing the expression induction conditions.

2 Materials and methods

2.1 Bacterial strain

A recombinant *E. coli* M15 strain containing pQE-30 expression vector with *cat1* gene was provided by the Institute of Bioactive Compounds, College of Sciences, Hue University. The *cat1* gene encoding protein CAT1 was isolated from *B. cepacia* DF4.

2.2 Cultivation of bacteria

The recombinant *E. coli* M15 strain was grown overnight in 5 mL of LB medium supplemented with 50 mg/L ampicillin and 50 mg/L kanamycin at 37 °C, 190 rpm. The overnight cultured cells (2.5 mL) were inoculated to a 250-mL flask containing 50 mL of LB medium with appropriate antibiotics. The cells were cultured at 37 °C and shaking speed 210 rpm until the cell density at OD_{600} reached an optimal value [11]. Cell density at OD_{600} (0.4–0.9), shaking speed (160–240 rpm), induction temperature (33–41 °C), induction time (4–12 hours), and IPTG concentration (0.6–1 mM) are the factors to be investigated to evaluate the expression of *cat1* gene [12]. The optimal results of the previously fixed conditions were used for the following assays.

2.3 SDS-polyacrylamide gel electrophoresis

After the cultivation of bacteria, the cells were harvested by centrifugation at 13000×g for 1 min. An equal volume of each sample (10 μL) was boiled at 95 °C for 10 min, then analyzed on 12% (w/v) SDS-PAGE. The gel was then stained with Coomassie Blue R-250 and observed under UV light. Image Lab software (ver 6.0.1, Bio-Rad) for densitometric analysis was used to estimate intensities of protein bands on gel [13].

3 Results and discussion

3.1 Effect of cell density at time of induction

Numerous studies have demonstrated that cell density at the time of induction has a significant effect on the synthesis and activity of recombinant proteins. Thus, it is necessary to investigate the cell density for high proteins before adding an inducer [14].

We investigated the effect of cell density at 6 different values of OD_{600} (0.4, 0.5, 0.6, 0.7, 0.8, and

pISSN 1859-1388
eISSN 2615-9678

0.9) on the expression of *cat1*. At each investigated cell density, the cells were induced to be expressed with 1 mM IPTG at 37 °C on a shaker at 180 rpm for 6 hours. The results reveal that *cat1* gene is successfully expressed as expected (~33 kDa) with different levels (Fig. 1).

The SDS-PAGE analysis shows that the recombinant CAT1 protein is overexpressed at a cell density of OD_{600} from 0.5 to 0.8. Under this condition, the production of *cat1* is strongest at OD_{600} 0.7, which is the optimal condition for the next experimental investigations. Although there is a slight difference in the expression levels between OD_{600} 0.7 and OD_{600} 0.8, OD_{600} 0.7 is a more practical value when this enzyme is produced on a large scale.

3.2 Effect of shaking speed at time of induction

To evaluate the effect of the shaking speed on enzyme expression, the shaking speeds from 160 to 240 rpm were tested during induction with 1.0 mM IPTG at OD600 0.7 at 37 °C for 6 hours.

The density of protein bands significantly increases when using different shaking speeds. The expression level of CAT1 enzyme reaches the highest value at the shaking speed of 200 rpm (Fig. 2). Thus, this optimal shaking speed is used for the next experiments.

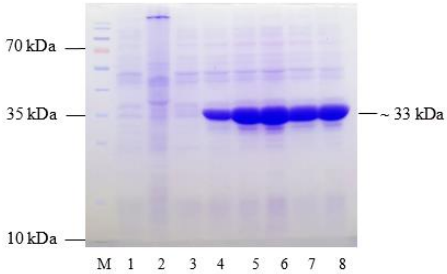

Fig. 2. Effect of shaking speed on the expression of cat1 gene in E. coli M15. M: protein weight standard (Thermo Scientific), 1: non-recombinant *E. coli* M15 cells, 2: B. cepacia DF4, 3: non-induced *E. coli* M15 cells, 4–8: expression of cat1 gene on a rotation shaker with a speed of 160–240 rpm, respectively.

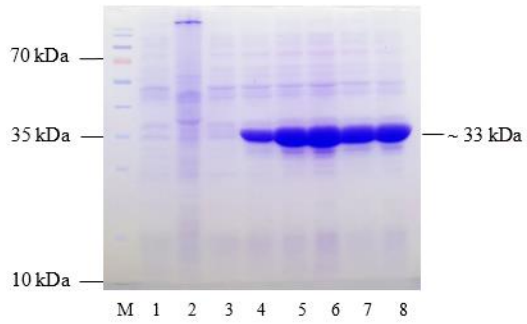

Fig. 1. Effect of shaking speed on the expression of cat1 gene in E. coli M15. M: protein weight standard (Thermo Scientific), 1: non-recombinant *E. coli* M15 cells, 2: B. cepacia DF4, 3: non-induced *E. coli* M15 cells, 4–8: expression of cat1 gene on a rotation shaker with a speed of 160–240 rpm, respectively.

3.3 Effect of induction temperature

Temperature is also considered to be one of the important parameters for the induction of recombinant protein expression [15]. The cultured cells were induced with 1 mM IPTG at different temperatures (33, 35, 37, 39, and 41 °C) at OD_{600} 0.7 and a shaking speed of 200 rpm for 6 hours.

The intensity of protein bands indicates that the highest expression of recombinant enzyme CAT1 was reached at 35 °C. Therefore, this temperature is used for further experiments (Fig. 3).

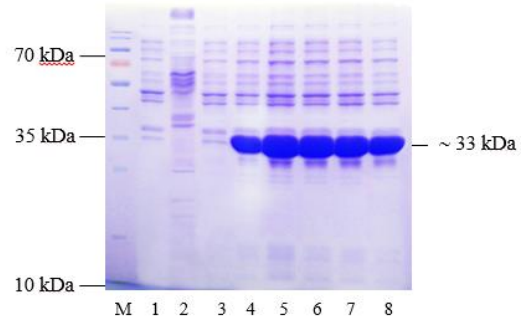

Fig. 3. Effect of induction temperature on the expression of cat1 gene in *E. coli* M15. M: protein weight standard (Thermo Scientific), 1: non-recombinant *E. coli* M15 cells, 2: B. cepacia DF4, 3: non-induced E. coli M15 cells, 4–7: expression of cat1 gene with induction temperature 33–41 °C, respectively.

Effect of induction time

Different studies have reported that induction time affects the expression of recombinant proteins in the bacterial expression systems [16, 17]. Therefore, the induction is performed during 4, 6, 8, 10, and 12 hours to investigate the *cat1* gene expression with 1.0 mM IPTG at 35 °C and 200 rpm. The results of SDS-PAGE indicate that the highest expression of catechol-1,2-dioxygenase occurs after 10 hours of induction (Fig. 4). Another study shows that the highest expression of the recombinant protein Rv1733 from *Mycobacterium tuberculosis* also occurs after 10 hours of induction with 0.4 mM of IPTG in *E. coli* BL21 [18].

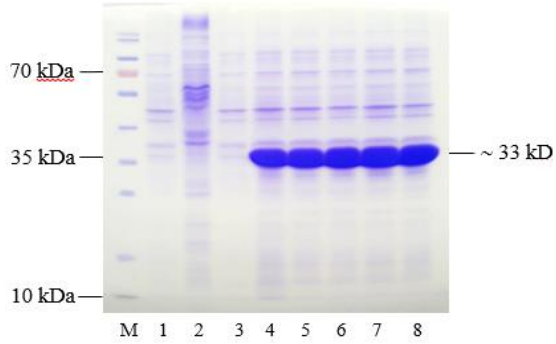

Fig. 4. Effect of induction time on the expression of cat1 gene in E. coli M15. M: protein weight standard (Thermo Scientific), 1: non-recombinant *E. coli* M15 cells, 2: B. cepacia DF4, 3: non-induced *E. coli* M15 cells, 4–7: expression of cat1 gene with an induction time of 4–12 hours, respectively.

3.4 Effect of IPTG concentration

IPTG toxicity promotes metabolic stress and reduces cell growth [12].

In this study, the expression of CAT1 was optimally induced with different IPTG concentrations (0.6, 0.8, 1.0, 1.2, and 1.4 mM) at a cell density of 0.7 (OD_{600}) in the shaking incubator (35 °C/200 rpm) for 10 hours. The results reveal that the IPTG concentration has an effect on the production of recombinant enzyme CAT1 in *E. coli* cells. Specifically, CAT1 exhibits high-yield expression at an IPTG concentration of 1.2 mM (Fig. 5).

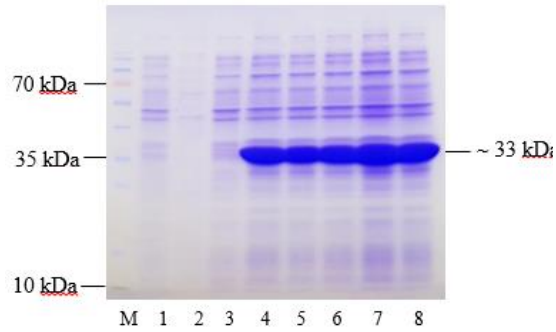

Fig. 5. Effect of induction inducer concentration on the expression of cat1 gene in E. coli M15. M: protein weight standard (Thermo Scientific), 1: non-recombinant *E. coli* M15 cells, 2: B. cepacia DF4, 3: non-induced *E. coli* M15 cells, 4–7: expression of cat1 gene with the IPTG concentration of 0.6–1.4mM, respectively.

4 Conclusion

In this study, we successfully optimize the conditions for *cat1* gene expression in terms of induction temperature, shaking speed, IPTG concentration, induction time, and cell density. These results could be applied to improve the biosynthesis of catechol 1,2-dioxygenase enzyme in a large-scale protein purification study.

Acknowledgements

This study was supported by College of Sciences, Hue University and Institute of Bioactive Compounds, College of Sciences, Hue University.

References

1. Thu NTT. Studying on the diversity of anaerobic bacteria community in bioremediated herbicide/dioxin cells [dissertation]. Hanoi: Vietnam Academy of Science and Technology; 2013.

pISSN 1859-1388
eISSN 2615-9678

2. Ministry of Natural Resources and Environment of Vietnam. Dioxin contamination in Vietnam: Emissions from industries and levels in the environment. Hanoi: Ministry of Natural Resources and Environment of Vietnam; 2014.

3. Montgomery JH. Groundwater Chemicals Desk Reference, 4th Ed. Florida (US): CRC Press; 2007. p. 328-330.

4. Thakore KN, Mehendale HM. Dibenzofuran. In: Wexler P, editor. Encyclopedia of Toxicology (3th Ed.). Oxford: Academic Press; 2014. p. 67-69.

5. Ali F, Hu H, Wang W, Zhou Z, Shah SB, Xu P, et al. Characterization of a dibenzofuran-degrading strain of *Pseudomonas aeruginosa* FA-HZ1. Environmental Pollution. 2019;250:262-273.

6. Hiraishi A. Biodiversity of dioxin-degrading microorganisms and potential utilization in bioremediation. Microbes and Environments. 2003; 18(3):105-125.

7. Giedraityte G, Kalėdienė L. Catechol 1,2-dioxygenase from α-naphthol degrading thermophilic *Geobacillus* sp. strain: purification and properties. Open Life Sciences. 2009;4(1):68-73.

8. Schlüter R, Lippmann R, Hammer E, Gesell Salazar M, Schauer F. Novel insights into the fungal oxidation of monoaromatic and biarylic environmental pollutants by characterization of two new ring cleavage enzymes. Applied Microbiology and Biotechnology. 2013;97(11):5043-5053.

9. Wang Z, Sun Y, Shi Y, Song W, Zhang C. Cloning, expression and characterization of a mesophilic catechol 1,2-dioxygenase from *Rhodococcus ruber* OA1. Science Alert. 2017;16(1):10-18.

10. Silva AS, Jacques RJS, Andreazza R, Bento FM, Roesch LFW, Camargo FAO. Properties of catechol 1,2-dioxygenase in the cell free extract and immobilized extract of *Mycobacterium fortuitum*. Brazilian Journal of Microbiology. 2013;44(1):291-297.

11. Zhang HB, Mao XQ, Wang YJ, Hu XQ. Optimization of culture conditions for high-level expression of dextransucrase in *Escherichia coli*. Journal of Food, Agriculture & Environment. 2009;7(3&4):75-78.

12. Ribeiro VT, Asevedo EA, Vasconcelos LTCP, Filho MAO, Araújo JS, Macedo GR, et al. Evaluation of induction conditions for plasmid pQE-30 stability and 503 antigen of *Leishmania i. chagasi* expression in *E. coli* M15. Applied Microbiology and Biotechnology. 2019;103:6495-6504.

13. Hariprasad G, Hariprasad R, Kumar L, Srinivasan A, Kola S, Kaushik A. Apolipoprotein A1 as a potential biomarker in the ascitic fluid for the differentiation of advanced ovarian cancers. Biomarkers. 2013;18(6):532-541.

14. Ma D, Ma C, Pan L, Li G, Yang J, Hong J, et al. Vaccination of chickens with DNA vacxin encoding *Eimeria acervulina* 3-1E and chicken IL-15 offers protection against homologous challenge. Experimental Parasitology. 2011;127(1):208-214.

15. Huong DT, Quy NT, Ha DTN, Hong LTT, Hai TN. Optimization of fermentation conditions for the expression of interleukin-3 in fusion with PelB in *E. coli*. Academia Journal of Biology. 2016;38(2):250-256.

16. Gopal GJ, Kumar A. Strategies for the production of recombinant protein in *Escherichia coli*. The Protein Journal. 2013;32(6):419-425.

17. Joseph BC, Pichaimuthu S, Srimeenakshi S, Murthy M, Selvakumar K, Genesan M, et al. An overview of the parameters for recombinant protein expression in *Escherichia coli*. Journal of Cell Science and Therapy. 2015;6(5).

18. Ashayeri-Panah M, Eftekhar F, Kazemi B, Joseph J. Cloning, optimization of induction conditions and purification of *Mycobacterium tuberculosis* Rv1733c protein expressed in *Escherichia coli*. Iranian Journal of Microbiology. 2017;9(2):64-73.