

Optimization of an organic solvents tolerance lipase expressed in *E.coli**

Ren Peng[†], Chun-Mei Tong and Ming-Qing Lin

College of Life Science, Jiangxi Normal University,
Nanchang, Jiangxi 330022, China

[†]E-mail: renpeng@jxnu.edu.cn

www.jxnu.edu.cn

Owing to the safety consideration and improvement of lipase productivity, the genes of lipase and its cognate foldase from *Pseudomonas aeruginosa* CS-2 was co expressed in *E.coli* BL2 (DE3) and the optimization of expression condition was investigated in the paper. The recombinant strain entered the stationary phase after 8h. The optimum conditions for the expression of intracellular lipase in *E.coli* BL21 (DE3) were induction time (6h), the concentration of isopropyl- β -thiogalactopyranoside (1.5mM) and induction temperature (25°C).

Keywords: lipase expression; *Pseudomonas aeruginosa*; organic solvents tolerance

1. Introduction

Lipases (glycerol ester hydrolyses E.C.3.1.1.3) catalyze different sorts of reactions such as hydrolysis, esterification, transesterification, aminolysis, acidlysis and alcoholysis [1]. The multifaceted features make lipase play a crucial role in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries [2]. Furthermore, lipase with organic solvents tolerance have capture tremendous attention due to their altered regiospecificity and stereoselectivity, higher solubility of substrate and ease of products recovery and ability to shift the equilibrium toward synthetic reaction, etc [3]. Until now several industrial processes are performed in the presence of organic solvents. However, organic solvents may inactivate lipase to some extent. Accordingly, more novel and organic solvent-tolerant lipases are required to deal with the harsh condition. In recent years a slice of organic solvent-tolerant lipases have been reported, which were produced from various species such as *Pseudomonas aeruginosa* LST-03, *Bacillus sphaericus* 205y, *Serratia marcescens* ECU1010,

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*Arthrobacter sp.*SD5, *Staphylococcus saprophyticus* M36, etc [4–8]. In our effort, an organic solvents tolerance lipase was isolated from *Pseudomonas aeruginosa* CS-2 [9]. Furthermore, owing to the safety consideration and/or improvement of lipase productivity, many microbial lipase genes were expressed in heterogeneous host such as *E.coli*, filamentous fungi and yeast [10–12].

In the present work, lipase and foldase from *Pseudomonas aeruginosa* CS-2 were coexpressed in *E.coli* BL21 (DE3). The optimization of expression condition was also investigated.

2. Materials and Methods

2.1 Bacterial strains, plasmid and reagents

The lipase and foldase gene from *Pseudomonas aeruginosa* CS-2 were cloned into plasmid pet28a. The recombinant plasmids, called pet28a-lip and pet28a-fol respectively were shown in Figure 1, which were harbored in *E.coli* Top10. Plasmid extraction kit was purchased from Tiangen Biotech (Beijing) Co. Ltd. P-NPP was purchased from Fluka Co.Ltd. All other chemicals were obtained from various commercial sources and were of analysis grade or higher grade.

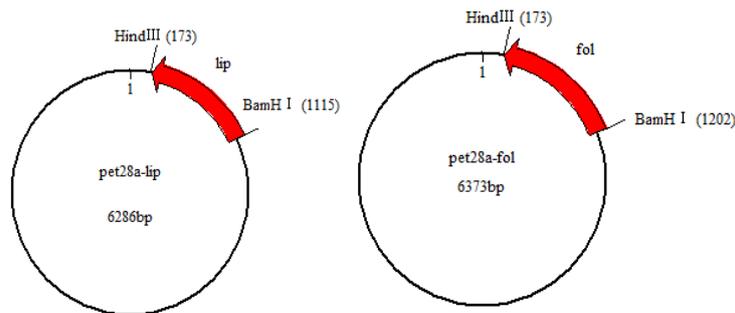


Fig. 1. The map of plasmid pet28a-lip and pet28a-fol

2.2 Co expression of pet28a-lip and pet28a-fol in *E.coli* BL21 (DE3)

The *E.coli* Top10 strains harboring pet28a-lip and pet28a-fol respectively were cultured in a LB medium containing kanamycin (50 µg/ml) at 37 °C with rotary shaking (190 r/min) overnight. The recombinant plasmid pet28a-lip and pet28a-fol were extracted by using plasmid extraction kit and were confirmed by agarose gel electrophoresis (Figure 2), which was subsequently transformed into *E.coli* BL21 (DE3) together. The positive clone was screened and then verified by colony PCR amplification. The positive clone was grown at 25 °C and

190r/min. After cultured for 3 hours, the strain was induced with isopropyl- β -thiogalactopyranoside for a certain interval at a certain temperature. The cells were harvested by centrifugation at 10,000 r/min for 10 min at 4 °C, which were resuspended in 50 mM Tris-HCl buffer. The mixtures were then disrupted by sonication and then centrifugated. The resultant supernatant was used for assay of lipase activity and protein concentration.

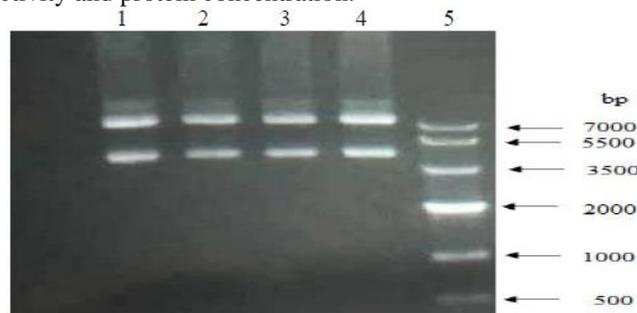


Fig.2. Agarose gel electrophoresis pattern of the extracted recombinant plasmid pet28a-lip and pet28a-fol. The recombinant plasmid pet28a-lip (lanes 1 and 2), the recombinant plasmid pet28a-fol (lanes 3 and 4) and DNA marker (lane 5), were run in agarose gel electrophoresis.

2.3 The growth curve of the positive clone

Bacterial growth was investigated by cultivating the positive clone at 25°C (190rev/min) in 50 ml of LB medium. Samples were harvested at a certain time intervals for determination of optical density at 600 nm.

2.4 The effect of induction time on the expression of intracellular lipase in *E.coli* BL21 (DE3)

In order to study the effect of induction time on the expression of intracellular lipase in *E.coli* BL21, the strain was induced with isopropyl- β -thiogalactopyranoside for a certain interval (2h, 3h, 4h, 5h and 6h).

2.5 The effect of concentration of isopropyl- β -thiogalactopyranoside on the expression of intracellular lipase in *E.coli* BL21 (DE3)

In order to study the effect of the concentration of isopropyl- β -thiogalactopyranoside on the expression of intracellular lipase in *E.coli* BL21, the strain was induced with isopropyl- β -thiogalactopyranoside (0.2mM, 0.5 mM, 1 mM, 1.5 mM and 2 mM) for a certain interval.

2.6 The effect of induction temperature on the expression of intracellular lipase in *E.coli* BL21 (DE3)

In order to study the effect of induction temperature on the expression of intracellular lipase expressed in *E.coli* BL21(DE3), the strain was induced with isopropyl- β -thiogalactopyranoside at a certain temperature (20°C, 25°C, 30°C, 33°C and 37°C) for a certain interval.

2.7 Determination of lipase activity

Lipase activity was assayed according to the method described by Peng et al [9].

2.8 Protein Assay

The protein concentration was determined by Bradford dye method using bovine serum albumin as a standard [9].

2.9 Statistical Analysis

Experimental data were presented as mean \pm SD. Statistical analysis was performed using SPSS 11.5 software.

3. Results and Discussion

3.1 The growth curve of the recombinant strain

Figure 3 showed the growth characteristics of the recombinant strain with regard to time. There was a rapid growth during 8 h of incubation, followed by a slow increase in the optical density at 600nm. It suggested that the recombinant strain entered the stationary phase after 8h.

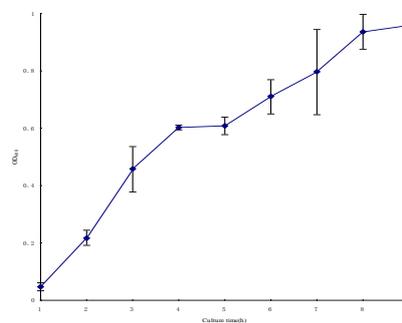


Fig. 3. The growth curve of the recombinant strain

3.2 The effect of induction time on the expression of intracellular lipase in *E.coli* BL21 (DE3)

The effect of induction time on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3) was indicated in Figure 4. The specific activity of intracellular lipase increased with the induction time from 2h to 3h. However, it is almost the same while the induction time is from 3h to 5h. The largest specific activity was achieved when the induction time is 6h. Generally, the functional lipase from *Pseudomonas aeruginosa* was dependant on its cognate foldase. The assay of intracellular lipase activity demonstrated that the co-expression of lipase and foldase in *E. coli* BL21 (DE3) gave rise to the formation of a functional lipase. In a similar effort, Wu *et al.* used two dual expression plasmid systems to overexpression of the lipase and its chaperone foldase from *Pseudomonas aeruginosa* in order to enhance the production of active lipase [13].

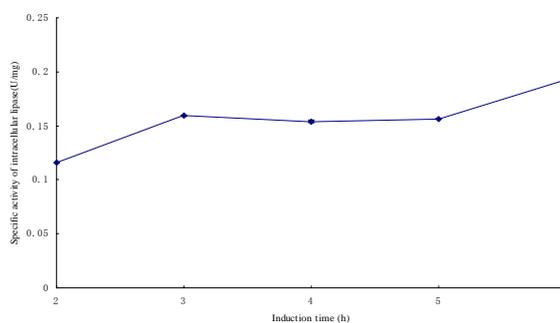


Fig. 4. The effect of induction time on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3)

3.3 The effect of the concentration of isopropyl- β -thiogalactopyranoside on the expression of intracellular lipase in *E.coli* BL21 (DE3)

The effect of the concentration of isopropyl- β -thiogalactopyranoside on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3) was indicated in Figure 5. Figure 5 showed the optimal concentration of isopropyl- β -thiogalactopyranoside was 1.5mM. Whereas the concentration of isopropyl- β -thiogalactopyranoside exceeded 1.5mM, the specific activity of intracellular lipase decreased. This reason was that excessive isopropyl- β -thiogalactopyranoside resulted in the formation of inclusion body, thereby reducing the quantity of the active protein.

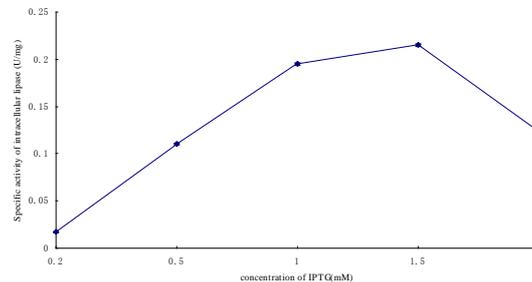


Fig. 5. The effect of the concentration of IPTG on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3)

3.4 The effect of induction temperature on the expression of intracellular lipase expressed in *E.coli* BL21 (DE3)

The effect of the concentration of induction temperature on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3) was indicated in Figure 6.

Figure 6 indicated that 25 °C was the best induction temperature for the expression of functional lipase. Generally speaking, the recombinant cells were cultured at a low temperature after the addition of IPTG to prevent the formation of inclusion bodies [14]. The study by Madan et al. indicated 25 °C is beneficial for lipase expression as compare with 30 °C and 37 °C [15].

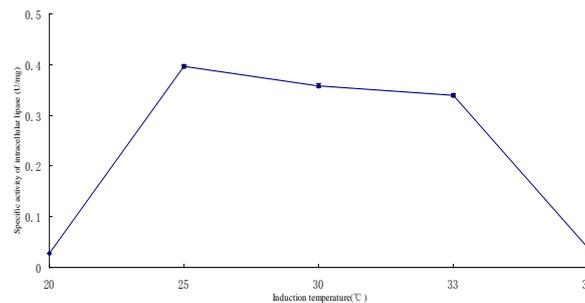


Fig. 6. The effect of induction temperature on the specific activity of intracellular lipase expressed in *E.coli* BL21 (DE3)

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