Original Article

An improved method for induction of competency in *Escherichia coli* DH5α

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Abstract

Efficient DNA transformation of bacterial competent cells has a critical role in biotechnology experiments such as gene cloning and protein expression. Transformation rates are commonly associated with the competency state of the bacterial cells. In this paper, it was demonstrated that transformation efficiency is strongly related to the growth phase of bacterial culture used for competent cell preparation, as well as special chemical treatments applied to make them competent. Results showed that exposure of the cells from the late growth phase (OD_{600}; 0.9-0.94) to monovalent and divalent cations resulted in the highest transformation efficiencies (about 100-fold). These competent cells are particularly useful for the transformation of ligated and large DNA molecules (more than 10 kbp).

Keywords: Monovalent cations, Competent cells, Transformation, *E. coli*

Introduction

Plasmid transformation into bacterial competent cells is a critical stage of gene manipulation in molecular cloning. In the early 1970s, Cohen [1] showed that recombinant plasmids can be assimilated by *E. coli* cells using a calcium chloride treatment. So far, several chemical methods have been developed for increasing the competency of *the E. coli.* A number of factors such as bacterial strain, medium composition, growth phase, both monovalent and divalent cations, and DNA size are important in the chemical transformation process [2]. Actively growing cells in early and mid-exponential phases are more susceptible to transformation [3]. Previous studies have reported that the cultures grown to mid-exponential phase (OD600 nm at 0.4) seem to give a high transformation rate for *E. coli* indicating the importance of cell growth phase [4]. Previous studies have shown that some species and strains are more susceptible to transformation compared to others due to differences in the cell wall and membrane structure [5]. *E. coli* K12 derivatives are the most popular *E. coli* laboratory strains for transformation [6]. The composition of media for transformation efficiency is less important [7]. Transformation efficiency decreases linearly with an increase in the plasmid size [8]. Prior studies have shown that the transformation efficiency of *E. coli* DH5α strains is stimulated by several cations such as Mg^{2+}, Mn^{2+}, Rb^{2+}, especially Ca^{2+}. Some monovalent cations also have been reported to lead to concentration-dependent induction of transformation [9]. In this investigation, cell densities of around 10^7–10^9 cells/mL, (OD600 nm at 0.3–0.4 and 0.9-0.94), corresponding to mid-exponential phase and late growth phases were treated with different compounds of monovalent–halogen with or without divalent cations that resulted in a concentration–dependent competency of the cells. It was found that our improved competent cells can raise transformation efficiency about 10^2 times especially for ligation products and large DNA molecules (more than 10 kb).

Materials and Methods

Bacteria, Plasmid, and Media

The *Escherichia coli* DH5α strain was employed for transformation. The plasmids were pBR322 (Amp^r, Tet^r) and ligation products of 5-12 kb. Cells were grown on Luria–Bertani (LB) and LB agar medium. The media were supplemented with appropriate antibiotics.

Chemical materials were purchased from Pharmacia Fine Chemicals Company (Uppsala, Sweden). The bacteria and plasmids were obtained from the National Institute of Genetic Engineering and Biotechnology (NIGEB; Tehran, Iran). All

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biochemical reagents were analytically pure. Plasmid DNA was extracted from *E. coli* by standard alkaline-lysis techniques [10].

Preparation of Competent Cell

10 µl of glycerol stock an *E. coli* DH5α was spread out on a fresh LB-agar plate and was grown overnight at 37 °C. A fresh single colony of the overnight culture was inoculated into 20-40 ml LB and was incubated overnight at 37°C by vigorous shaking. The overnight culture was diluted 1:20 with fresh LB and was shaken at 37°C until reaching the $O.D_{600}$ of 0.94. The culture was ice-bathed for 20 min. The cells were harvested by centrifugation at 4000 rpm, at 4°C, for 10 min, and were gently re-suspended in 3 ml of ice-cold washing buffer I (Table 1) and were kept in ice for 10 min. The cells were harvested again by the same centrifugation. The supernatant was discarded, the pellet was gently resuspended in 3 ml of ice-cold washing buffer II (Table 1), and was kept in an ice bath for 10 min. The cells were collected by centrifugation at 3000 rpm, at 4°C, for 10 min. The supernatant was discarded and the pellet was gently resuspended in 1 ml of ice-cold storage buffer (Table 1). The cells were expected to become competent after overnight incubation (16 h) in the ice. All pipette tips used during the experiments were also chilled. The standard $CaCl_2$ treatment for competent cell preparation was also used for comparative analysis of the results [11].

DNA Transformation

10 µl of plasmid DNA (5µg) was mixed with 100 µl of competent cell suspension and was incubated in an ice bath for 30 min. The mixture was heat-shocked in a water bath at 42℃ for 90-100 seconds and was rapidly transferred to an ice bath for 3-5 min. 400µl of LB medium was added and the mixture was incubated at 37℃ for 45-60 min.

Evaluation of Transformation Efficiency

25 µl of each sample was spread onto a selective LB agar plate containing the appropriate antibiotic. The plates were incubated at 37℃ for 18–20 h, and then the number of CFU (Colony Forming Units) formed per microgram of DNA was calculated.

Statistical Analysis

All the experimental data values were obtained from three independent experiments, and the results were presented as mean ± Standard Error of Mean (SEM). Statistical analyses were performed using SPSS software Ver.16.0 [12].

Results and Discussion

Effect of Cell Growth Phase on Cell Competency

It has been previously reported that the state of the cell culture is important for transformation efficiency. Cell densities around 10^7–10^8 cells/mL (OD_{600} at 0.4), corresponding to the mid-exponential phase are considered as optimum for *E. coli* [13]. In this study, the *E. coli* DH5α cells were harvested at the OD_{600} range of 0.25-1.25 for competent cell preparation and transformation using the standard $CaCl_2$ method. Competent cells were transformed with pBR322 plasmid DNA and were assayed for transformation and viability. Results showed that the OD_{600}, of 0.35 and 0.95 yielded a higher frequency of transformation (Fig.1). Re-examination of cell culture resulted in 6×10^5 and 7×10^6 colony forming units per microgram of transforming DNA (CFU/µg) for cells harvested at the OD_{600}, of 0.33 and 0.94, respectively. Most studies have investigated the effect of a limited range of OD_{600}, usually around the OD_{600}, of 0.45 on transformation efficiency [14]. The results of this research showed that transformation efficiency could also be enhanced at high cell densities (i.e., OD_{600} of 0.94).

This may be resulted from the cooperative transformation of the natural and artificial competency state of *Escherichia coli*. Some studies have shown that *Escherichia coli* cells harvested from stationary phase are able to uptake DNA from the environment [15]. Although, specific inducing signals for DNA uptake have not been known clearly, but nutritional state can be one of transformation regulatory machinery factors. Previous studies showed that, in laboratory conditions, usually natural competence is tightly regulated by nutritional shortages or adverse conditions [16]. Our results also showed that, competent cells made from bacterial cultures in stationary phase give larger colonies compared to the competent cells prepared from cultures with the OD_{600} of 0.4 (log phase) (Fig. 2). This property may also be attributed to 1) cell nutrition states (nutritional shortage state) which have a much tendency to over-eat resulting in nutrient and DNA uptake. Therefore, physiological state of the cells and probable natural transformation may contribute to high efficiency of our transformation, and 2) membrane of the *E. coli* which has hundreds of pores (adhesion zones) during rapid growth of the bacterial cells, as we postulated some of pores to be integrated to each other because of increasing number of pores at the end of log phase, as a result, they can absorb ligation product or large DNA.

Effect of Treatment Buffer on Competency

Composition and concentration of cations, such as Mg^{2+}, Mn^{2+}, Rb^+, K^+ and especially Ca^{2+} in treatment buffer are other important factors influencing the competency of bacterial cells [3]. The calcium chloride ($CaCl_2$) method has been introduced as a popular efficient procedure, however, it is not enough effective, especially for the transformation of ligation product and large size DNA. In the present study, several improvements were employed to the treatment buffer (0.1M $CaCl_2$) by replacing with different compositions and concentrations of monovalent and divalent cations (Table 1) to obtain efficient competent cells. Table 1 indicates transformation efficiency for different treatment buffers using pBR322 plasmid vector. Results can be summarized as follows: treatments II, III, and IV were 100-fold, 25-fold, and 10-fold more efficient than the standard $CaCl_2$ method, respectively. For treatment I, efficiency was 40-fold less than $CaCl_2$ method.

The hypotheses proposed for explanation of our results are as follows: 1) in this work, transformation efficiency was high when the cells were treated with storage or final treatment buffer containing monovalent cation and $CaCl_2$. Prior studies showed that, presence and formation of poly-β-hydroxybutyrate/calcium polyphosphate (PHB/Ca^{2+}/PPi) complexes and divalent cations such as Ca^{2+}, Mn^{2+}, Sr^{2+}, or Mg^{2+} are essential in genetic competence. The complexes are assumed to form channels or disintegrated lipid bilayer of the plasma membrane facilitating DNA transfer, and divalent cations increase penetration of the outer membrane and/or linking of DNA to PPi at the mouth of the channel complex by cross-linking phosphate groups of the complexes and DNA polymer. Huang and Reusch (1995) suggested critical selectivity of PHB/PPi for Ca^{2+} in the complexes; however, Das et al. [17] proposed that other divalent cations such as Sr^{2+}, Ba^{2+}, and Ca^{2+} could participate in the formation of complexes. They investigated the effect of monovalents, divalents and also the simultaneous effect of several cations and found high transformation efficiency for the competence-inducing buffer with three cations, Ca^{2+}, Mn^{2+}, and K^{2+} (10mM, 45mM, and 100mM, respectively).

Considering our results, we postulate that, calcium may participate in complex formation and potassium-mediated cell- membrane disintegration or DNA to PPi at complexes. We also assumed that the potassium with ionic radius bigger than calcium could produce large channel gate or cause cell membrane disintegration for transferring large size DNA. On the other hand, sufficient values of two cations with different roles induce the mentioned transformation process with no interference. Sufficient O/N incubation of cells at the last stage of preparation of competent cells is also effective. Sarkar et al. [18] indicated that $CaCl_2$ mediates binding interaction between naked DNA plasmid with negatively charged lipopolysaccharide (LPS) molecules. Regarding transformation, they assumed transfer of DNA molecules across the least-barrier path at zones of adhesion, where the outer and inner cell membranes fuse with the pores in the cell wall, which are rich in negatively charged lipopolysaccharide (LPS) molecules. We believe that the potassium ions with a big radius can produce big shield around negatively charged DNA and help in transferring DNA molecules into the cell without much change in pH of cell medium.

In relation to the preference of washing buffer to the composition of KI/KCl over KCl, seemingly it is due to weak acidities of HI relative to HCl produced from KCl and KI breakup. HI is a weak acid and slightly changes the pH of the cell medium with the least effect on cell viability.

Conclusion

The results of this study indicated that competency and DNA transformation of *E. coli* could be further increased by employing a combination of monovalent and divalent cations for the preparation of competent cells from bacteria in the late log phase of cell growth. Therefore, modifications in the $CaCl_2$ method presented in this study provide a more efficient *E. coli* transformation with great importance in genetic engineering and biotechnology.

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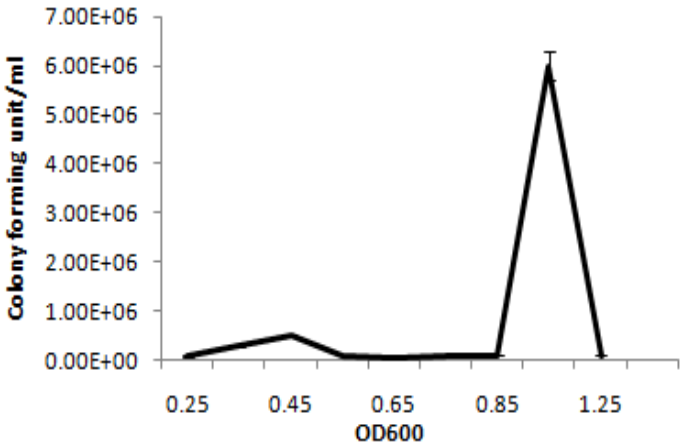

Figure 1. Transformation of competent *E. coli* DH5α cells derives from various stages of growth.

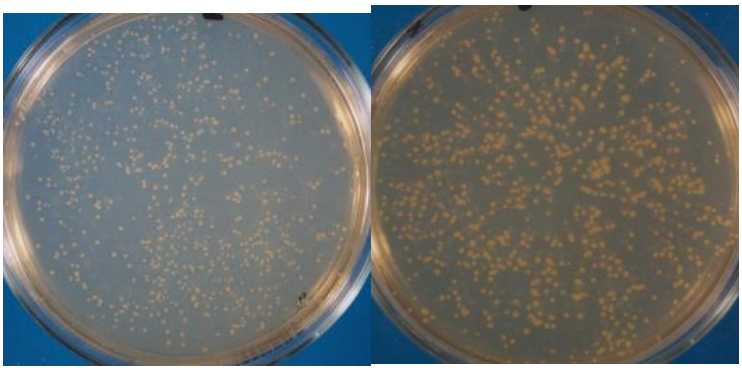

Figure 2. *Comparison* of *colony size* of *E. coli* transformants obtained from cultures with OD_{600} of 0.33 (Left) and 0.94 (Right).

Table 1. A comparison of different treatment composition and concentration for competency experiment.

Treatment	Washing Buffer I		Washing Buffer II		Storage Buffer[1]		Efficiency of transformation (CFU/µg DNA)
	Component	Concentration (mM)	Component	Concentration (mM)	Component	Concentration (mM)	
Treatment I	KCl	150	KCl	100	KCl	150	$(2.3\pm1.1)\times10^5$
	KI	100	KI	50			
			NH_4Cl	50			
Treatment II	KCl	150	KCl	80	KCl	80	$(8.2\pm2.1)\times10^8$
	KI	100	KI	80	$CaCl_2$	80	
Treatment III	KCl	150	KCl	150	KCl	80	$(2\pm1.8)\times10^8$
					$CaCl_2$	80	
Treatment IV	KCl	150	KCl	150	$CaCl_2$	100	$(8.3\pm4.9)\times10^7$
	$MgCl_2$	100	$MgCl_2$	100			
Treatment V	$CaCl_2$	100	$CaCl_2$	100	$CaCl_2$	100	$(8.1\pm5.6)\times10^6$

[1] The storage buffer contains the same volume of ice-cold 40% glycerol and cation(s).