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Optimizing Bacterial Transformation in the pDNA-Cationic Lipid - Surfactant Complex Systems by Adjusting $CaCl_2$ Concentration and Incubation Temperature

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Optimizing Bacterial Transformation in the pDNA-Cationic Lipid - Surfactant Complex Systems by Adjusting $CaCl_2$ Concentration and Incubation Temperature

Summary : *In this study effects of $CaCl_2$ concentration and incubation temperature on the E. coli transformation by plasmid DNA complexes were investigated. Three pDNA formulations (pDNA, pDNA + Zwittergent® 3-08, pDNA: Cationic Lipid (1:1w/w) + Zwittergent® 3-08) were examined. The formulation containing plasmid DNA : cationic lipid complex (1:1 w/w) and Zwittergent® 3-08 showed better transformation efficiency. 50 mM $CaCl_2$ concentration and 37-40°C incubation temperatures showed positive effects on the number of transformed E. coli cells.*

Key Words: *plasmid DNA; cationic lipids; zwitterionic surfactants; bacterial transformation; E. coli*

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pDNA - Katyonik Lipid - Surfaktan Kompleks Sistemlerinde $CaCl_2$ Konsantrasyonunun ve İnkübasyon Sıcaklığının Ayarlanmasıyla Bakteri Transformasyonunun Optimizasyonu

Özet : *Bu çalışmada, $CaCl_2$ konsantrasyonu ve inkübasyon sıcaklığının plazmid DNA kompleksleri ile E. coli transformasyonu üzerindeki etkileri araştırılmıştır. Üç plazmid DNA formülasyonu (pDNA, pDNA + Zwittergent® 3-08, pDNA: Katyonik Lipid (1:1a/a) + Zwittergent® 3-08 araştırılmıştır. Plazmid DNA : katyonik lipid kompleksi (1:1 a/a) ve Zwittergent® 3-08 içeren formülasyon, en iyi transformasyon etkinliğini göstermiştir. 50 mM $CaCl_2$ konsantrasyonu ve 37-40°C inkübasyon sıcaklıklarının transforme olmuş E. coli hücre sayısı üzerinde olumlu etkiye sahip olduğu belirlenmiştir.*

Anahtar kelimeler : *plazmid DNA; katyonik lipidler; zwitteriyonik surfaktanlar; bakteri transformasyonu; E. coli*

INTRODUCTION

Transformation is an important step in DNA cloning, which is one of the most important components of recombinant DNA technology and many other areas of molecular biology[1]. Since transformation of E. coli is an essential step in many cloning experiments, it is desirable that it should be as efficient as possible[2]. Most types of bacteria cannot take up DNA efficiently unless they have been exposed to special chemical or physical treatments to make them permeable[3]. There are two main techniques for transforming bacterial cells. The first and perhaps the most commonly used method is to make bacteria 'competent' by treating with a chemical such as $CaCl_2$ to make the bacterial cell wall permeable to DNA transfer[4,5]. Several groups of workers have examined the factors affecting the efficiency of transformation. It has been found that E. coli cells and plasmid DNA can interact effectively in an environment of calcium ions and low temperature (0-5°C), and that a subsequent heat shock (37-45°C) is useful, but not strictly necessary[6-9]. Several other methods, especially the inclusion of metal ions in addition to calcium, have been shown to improve the process[10].

The other method of transforming bacteria is electroporation, which requires special expensive equip-

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ment and has poor reproducibility[11]. For years, electroporation has been an effective method for introducing DNA into bacteria, yeast, mammalian cells and plant protoplasts. Electroporation involves the application of an electric field creating pores or holes in the membranes of cells through which DNA is driven as a result of its negative charge[12-16].

The aim of our study was optimization of a transformation method for recombinant DNA technology by adjusting $CaCl_2$ concentration and incubation temperature of bacterial growth in the systems of plasmid DNA complexed with cationic lipids and zwitterionic surfactants.

MATERIALS AND METHODS

MATERIALS

ESCORT™, a lipid suspension, consisting of 2 mg/mL DOTAP/DOPE [(N (1- (2, 3-dioleyloxy) propyl) - N, N, N, trimethyl ammoniumbromide)/(Dioleylphosphatidyl ethanolamine)] (1:1 w/w) was obtained from Sigma Chemical Company. Zwitterionic surfactant Zwittergent® 3-08 was obtained from Calbiochem. 3 kb, Escherichia coli pBluescribt plasmid was selected as the model vector which carries an ampicillin resistance gene and β-galactosidase gene alpha- subunit (Stratagene).

METHODS

Parameters Evaluated for Transformation Efficiency of E. coli

For optimization of transformation three different formulations, four different bacterial growth temperatures and three different $CaCl_2$ concentrations were selected. Parameters evaluated for transformation efficiency of E. coli are seen in Table 1.

Plasmid DNA Isolation

Plasmid DNA pBluescribt, was selected as a model vector which carries an ampicillin resistance gene and β-galactosidase gene α-subunit. The plasmids were amplified in XL-1 Blue strain of Escherichia coli. Bacteria containing plasmids were grown overnight, plasmids were isolated by alkaline lysis method[4] and purified using multiple phenol/chloroform extractions. DNA was then precipitated by ethanol and centrifuged. After the removal of the supernatant, the pellet was dried and dissolved in 50 µL Tris-EDTA (TE) buffer which contains 10 mM Tris pH 7.4, 1mM ethylendiaminetetraacetic acid (EDTA) and RNase at a concentration of 20 $\mu g.mL^{-1}$. The plasmids were stored at -20 °C until use. DNA concentrations were measured by UV absorption at 260 nm, and purity was confirmed by 1% agarose gel electrophoresis[4].

Preparation of Transformation Systems

Complexes used for transformation of bacteria were prepared by mixing buffer solutions of pBluescribt plasmid with the lipid reagent that contains cationic lipid DOTAP and zwitterionic neutral helper lipid DOPE at a ratio of 1:1 (w/w) and zwitterionic surfactant at minimum inhibitor concentration of 3.75 % (w/v). ESCORT™ lipid suspension was the lipid mixture, and Zwittergent® 3-08 (Z-8) solution was used as the surfactant. Naked plasmid DNA and plasmid DNA mixed with only zwitterionic surfactant Z-8 without lipid mixture were also used in transformation studies.

Transformation Studies

Escherichia coli XL-1 Blue cells and pBluescribt plas-

Table 1. Parameters Evaluated for Transformation Efficiency of E. Coli

Formulations		Bacterial Growth Temperature	$CaCl_2$ Concentrations (mM)
Formulation	Code		
		25°C	10 50 100
(A) Naked pDNA	pDNA	30°C	10 50 100
(B) pDNA + Zwittergent® 3-08	pDNA + Z-8	37°C	10 50 100
(C) pDNA:Cationic lipid (1:1 w/w) + Zwittergent® 3-08	pDNA:L (1:1w/w) + Z-8	40°C	10 50 100

mid which contains a gene for Ampicillin resistance were used. A single colony of XL-1 Blue was used to inoculate 15 mL of Luria-Bertani (LB) broth growth medium and grown overnight at 25°C, 30°C, 37°C and 40 °C in a shaking incubator. After 5 minutes centrifugation of the overnight culture at 4000 rpm, pellets were resuspended in 2 mL of 10 mM, 50 mM and 100 mM $CaCl_2$ solutions. 50 µL of bacterial suspension was added to 4.8 µg / 50 µL pDNA samples and after incubation for an hour at 37°C, samples were washed twice with 5% (w/v) BSA solution by centrifugation. Pellets were suspended in 100 µL of LB medium and inoculated on LB agar containing 75µg/mL ampicillin after incubating at 37°C for one hour. The transformed colonies were counted either after 24 hours or 48 hours incubation periods.

RESULTS AND DISCUSSION

Formulation Effects

Three plasmid DNA formulations (pDNA, pDNA + Zwittergent® 3-08, pDNA: Cationic Lipid (1:1w/w) + Zwittergent® 3-08) were investigated regarding their transformation efficiencies. Bacterial transformation could not be achieved by using naked pDNA and the least efficient transformation was observed by using plasmid DNA:Zwittergent® 3-08 mixture. Among these three formulations, plasmid DNA : cationic lipid complex (1:1 w/w) and Zwittergent® 3-08 complex formulation was determined as the most efficient mixture. Complex formulations were prepared by using different amounts of cationic lipids. Optimal lipid - DNA ratio in complex formation was determined according to the results of gel retardation assay, DNase I protection assay and bacterial transformation. By increasing the amounts of lipids in the complex formulations, protection of DNA against DNase I enzyme was achieved. Although all complex formulations could be transformed, due to the cell toxicity of the lipids, transformation efficiency is decreased over 1:1 ratio. After selecting the best pDNA : cationic lipid ratio which was 1:1 w/w (Figure 1), zwittergent was incorporated into the system by considering their MIC values.

Optimum $CaCl_2$ Concentration

Effect of the 10 mM, 50 mM and 100mM $CaCl_2$ concentrations on the bacterial transformation was studied. As seen from Figure 2-A, only a few bacteria can be transformed with pDNA at 10 mM $CaCl_2$ concentration whereas at 100 mM $CaCl_2$ concentration which is very high, transformant bacterial cells are less than at 50 mM $CaCl_2$ levels (Figure 2-C). The optimum $CaCl_2$ concentration for an efficient transformation was found to be 50 mM (Figure 2-B). Factors such as plasmid characteristics, amount, purity, cell density and marker all influence the transformation efficiency. Transformation efficiency can be affected by the Ca^{2+} induced phosphatidylethanolamine mechanism[8]. Here $CaCl_2$ concentration is a critical parameter in the phosphoethanolamine dependent membrane enyzme activity.

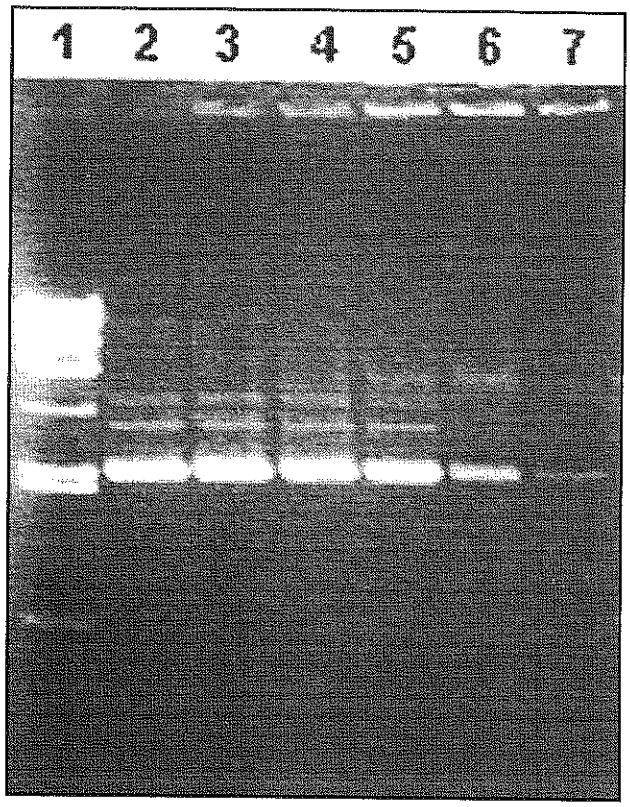

Figure 1. Gel Retardation Assay
Lane 1: λ DNA Hind III Digest MW Marker
Lane 2: Plasmid DNA
Lane 3: Plasmid DNA:Lipid → 1: 0,125 w/w
Lane 4: Plasmid DNA:Lipid → 1: 0,25 w/w
Lane 5: Plasmid DNA:Lipid → 1: 0,5 w/w
Lane 6: Plasmid DNA:Lipid → 1: 1 w/w
Lane 7: Plasmid DNA:Lipid → 1: 2 w/w

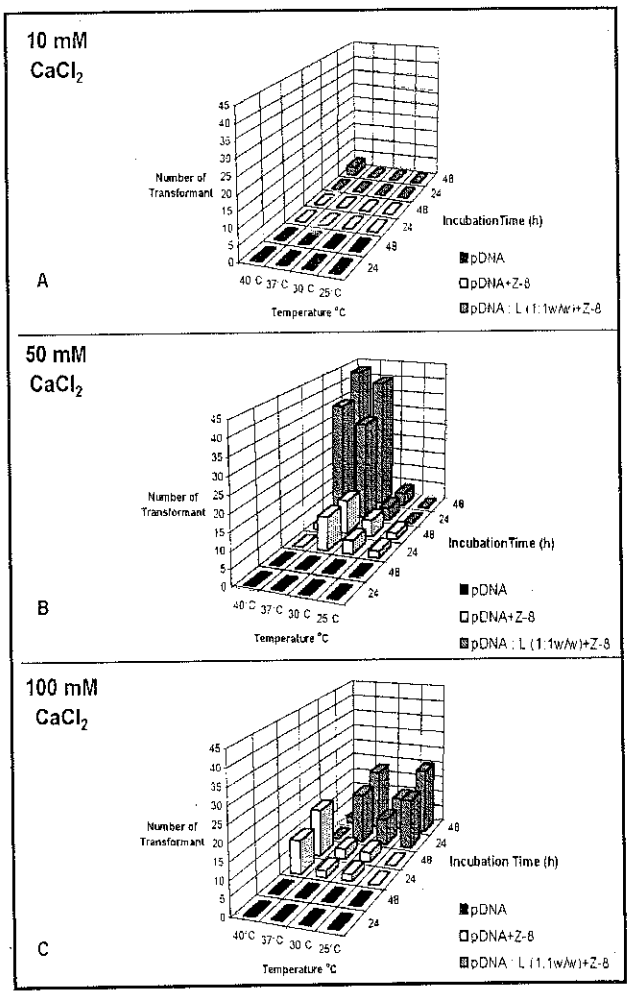

Figure 2. Effect of $CaCl_2$ concentrations on transformation efficiency of formulations which were transformed to bacteria grown at different temperatures.
(A) 10 mM $CaCl_2$ concentration
(B) 50 mM $CaCl_2$ concentration
(C) 100 mM $CaCl_2$ concentration

Optimum Incubation Temperature

Incubation temperature is also an important factor in the transformation efficiency of E. coli cells. Among the temperatures investigated, 25°C was found least efficient as transformation temperature for 10 mM and 50 mM concentrations (Figure 3). 30°C, 37°C and 40°C temperatures were found suitable for transforming bacteria for 50 mM and 100 mM concentrations. In Figure 3 the transformation profile of bacteria grown at different temperatures is seen. $CaCl_2$ concentration and composition of the pDNA formulation have additional effects on the incubation temperatures.

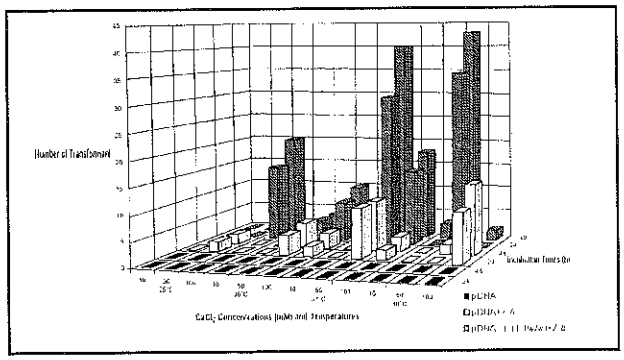

Figure 3. Effect of different $CaCl_2$ concentrations and bacteria incubation temperatures on transformation efficiency.

By considering all the factors, 50 mM $CaCl_2$ concentration, plasmid DNA : cationic lipid (1:1 w/w) + Zwittergent® 3-08 complex formulation and 37°C - 40°C temperatures seem to be productive conditions for optimal transformation studies.

CONCLUSION

It can be concluded from the results that cationic lipid and surfactant mixtures and optimized $CaCl_2$ concentration and temperature conditions may provide an efficient way for transforming bacteria with pDNA. The positively charged lipids can attach to pDNA, and also bind to cells to simplify DNA transfer into the cells. Cationic lipids have been used for transfection of eucaryotic cells for years, but in this study it has been established that these systems are also useful for transformation of procaryotic cells. $CaCl_2$ concentration and incubation temperatures are important parameters in optimising bacterial transformation.

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