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RESEARCH ARTICLE

An Alternative Rapid Method for Transformation of *Escherichia coli*

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An Alternative Rapid Method for Transformation of Escherichia coli

Summary

Transformation of bacteria with plasmids is widely used for molecular cloning of the genes and other purposes. To enable naked DNA to enter the cells, either bacteria are made "competent" by treatment with calcium chloride, or electroporation, which requires expensive equipment, is used. As an alternative to these methods, we have investigated cationic lipids and zwitterionic surfactants for their ability to carry plasmids into bacterial cells. For transformation studies, overnight Escherichia coli cultures were suspended in calcium chloride solution; naked or complexed plasmids with lipids and surfactants were mixed with prepared bacteria and incubated either on ice or at 37° C for various time periods. After washing the samples with phosphate buffer (pH 7.4), Luria Bertani (LB) medium or 5% (w/v) bovine serum albumin (BSA) solutions, pellets were inoculated on LB agar. Among the factors evaluated for transformation of bacteria, the best results were obtained with the complex DNA-cationic lipid-zwitterionic surfactant, incubation on ice for one hour, and treatment with BSA wash solution. Complexing plasmids with lipids and surfactants and washing with BSA solution may promise easy and rapid means to transform various types of bacteria.

Key Words : *Bacterial transformation, cationic lipids, Escherichia coli, plasmid DNA, zwitterionic surfactants.*

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Escherichia coli Transformasyonu için Altrenatif Hızlı Bir Yöntem

Özet

Bakterilerin plazmitlerle transformasyonu genlerin moleküler klonlanması ve diğer amaçlar için sıklıkla kullanılmaktadır. Çıplak DNA'yı hücrelere sokabilmek için bakteri kalsiyum klorür ile muamele edilerek, "kompetan" hale getirilir veya pahalı ekipman gerektiren elektroporasyon kullanılmaktadır. Biz bu yöntemlere alternatif olarak katyonik lipitlerin ve zwitteriyonik sürfaktanların plazmitleri bakteri hücrelerine taşıyabilme yeteneklerini araştırdık. Transformasyon çalışmaları için, bir gecelik Escherichia coli kültürleri kalsiyum klorür çözeltisi içerisinde süspande edilmiş; çıplak veya lipit ve sürfaktanlarla kompleksleştirilmiş plazmitler hazırlanmış bakteri ile karıştırılmış ve buz üzerinde veya 37° C'de çeşitli zaman aralıklarında inkübe edilmiştir. Örneklerin fosfat tamponu (pH 7.4), Luria Bertani (LB) besiyeri veya %5 (a/h) sığır serum albumini (BSA) çözeltileriyle yıkanmasından sonra, pelletler LB agar üzerinde inoküle edilmiştir. Bakteri transformasyonu açısından değerlendirilen bu faktörler arasında en iyi sonuçlar DNA-katyonik lipit-zwitteriyonik sürfaktan kompleksi, buz üzerinde bir saat inkübasyon ve BSA yıkama çözeltisi ile muamele edilerek elde edilmiştir. Plazmitlerin lipitlerle ve sürfaktanlarla kompleksleştirilmesi ve BSA çözeltisi ile yıkanması çeşitli bakteri tiplerini transforme etmek için kolay ve hızlı yollar olarak umut vericidir.

Anahtar Kelimeler : *Bakteri transformasyonu, katyonik lipitler, Escherichia coli, plazmit DNA, zwitteriyonik surfaktanlar.*

INTRODUCTION

Recombinant DNA technology is an effective way of creating organisms with specific genetic attributes. With the discovery of DNA transformation, in which a gene from one type of cell can be transferred to another type of cell, prokaryotic or eukaryotic systems were developed as useful expression systems for various purposes in molecular biology and biotechnology[1]. Although transformation experiments in bacteria were started much earlier, transfer of genes into eukaryotic cells for gene therapy has

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become an area of interest in recent years. Viral and non-viral gene delivery systems and a variety of methods have been developed to accomplish gene transfer into eukaryotic cells. These techniques involve the direct physical introduction of genetic material into cells by electroporation[2-6], microinjection[7,8], particle bombardment[9-13], or the disruption of cell membranes to allow the transfer of DNA using genetically modified viruses[10,14,15] and non-viral DNA complexes with polycations[12,16-20] or lipids[10,18,21-28].

Transformation of foreign genetic materials into cells by lipids has become a common technique for mammalian cell systems, but transformation efficiency of these carriers for bacteria and yeast cells has not been fully investigated[16].

In our study, we evaluated the transformation efficiencies of cationic lipid-plasmid DNA complex formulations with prokaryotic systems.

There are two main techniques for transforming bacterial cells. The most commonly used method is preparing "competent" bacteria by treating with $CaCl_2$ to make their cell wall permeable to DNA[2]. This process takes a few days of preparation and researchers prefer to purchase previously prepared competent cells, which should be shipped frozen. Electroporation is the second means of transforming bacteria, and this requires special expensive equipment and has poor reproducibility[3, 5, 6, 9, 10, 29, 30].

As an alternative to the known transformation methods, we have developed a highly efficient, rapid, relatively inexpensive and versatile DNA delivery technique, which can be used with a variety of prokaryotic cell types and eliminates the use of the standard transformation protocol.

MATERIALS and METHODS

MATERIALS

Lipid suspension, consisting of 2 mg/mL DOTAP / DOPE [(N (1-(2, 3 - dioleyloxy) propyl) - N,N,N, trimethyl ammonium bromide) / Dioleoyl phosphatidyl ethanol amine)] (1:1 w/w) (ESCORT™) was obtained from Sigma Chemical Company (St. Louis, USA). Zwitterionic surfactant Zwittergent®3-08 (n-octyl-N,N-dimethyl-3-ammonio-1-propane sulfonate) was obtained from Calbiochem (USA). Lambda DNA, Hind III-digested, DNA fragment size marker and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (St. Louis, USA). Escherichia coli pBluescript® plasmid (3 kb) was obtained from Stratagene (USA). All other chemicals used were reagent grade (Sigma Chemical Company, St. Louis, USA).

METHODS

Plasmid DNA Isolation

Plasmid DNA pBluescript®, which carries an ampicillin resistant gene and β-galactosidase gene α-subunit, was selected as a model vector. The plasmids were amplified in XL-1 Blue strain of *Escherichia coli*. Bacteria containing plasmids were grown overnight, and the plasmids were isolated by alkaline lysis method[2] 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, 1 mM ethylenediaminetetraacetic acid (EDTA) and RNase at a concentration of 20 µg/mL. Plasmids were stored at –20˚C until used. DNA concentrations were measured by UV absorption at 260 nm, and purity was confirmed by 1% agarose gel electrophoresis[2].

Preparation of Lipid-DNA Complexes

Complexes were prepared by mixing TE buffer solutions of the pBluescript® plasmid with the lipid reagent at various concentrations (1:0; 1:0.125; 1:0.25; 1:0.5; 1:1; 1:2 w/w plasmid DNA:lipid). Lipid reagent contained cationic lipid DOTAP and zwitterionic neutral helper lipid DOPE at a ratio of 1:1 (w/w). The time required for complex formation was then checked by running the samples on 1% agarose gel.

Determination of Optimal Lipid-DNA Ratios in Complex Formation

In this part of the study, optimal plasmid DNA:lipid ratio was selected by using the gel retardation assay, DNase I protection assay and classical bacterial transformation method.

Gel Retardation Assay

The formation of complexes between cationic lipids and plasmid DNA was monitored by gel retardation assay. Samples were run at constant voltage (60v) on 1% agarose gel with Tris-acetate-EDTA (TAE) buffer. The gel was then stained for 30 min in ethidium bromide solution at a concentration of 1 µg/mL and photographed with UV transilluminator.

DNase I Protection Assay

The effect of complex ratios on protection of DNA from DNase I degradation was evaluated by preparing cationic lipid-plasmid complexes. Samples of 5 µg pDNA-containing complexes were mixed with 50 µL of 100 Kunitz U /mL DNase I and incubated at 37˚C for 15 min. The reaction was stopped with 100 µL of 0.1M EDTA (pH:8) and then extracted twice with phenol-chloroform mixture to remove lipid material. DNA in the aqueous layer was precipitated with ethanol and subjected to agarose gel electrophoresis to determine DNA integrity[31].

Bacterial Transformation

Escherichia coli XL-1 Blue cells were selected as a model transformation system for the development of efficient cationic lipid – pDNA complexes. XL-1 Blue cells were produced using the plasmid pBluescript®, which contains a gene for ampicillin resistance. A single colony of XL-1 Blue was used to inoculate 10 mL of LB medium and grown overnight at 37˚C in a shaking incubator. The 50 µL of overnight culture was then subcultured to 10 mL of fresh medium and incubated for two more hours. After centrifugation of the culture, pellets were suspended with 1.5 mL 50 mM $CaCl_2$ and incubated on ice for an hour. After centrifugation, pellets were suspended with 1.5 mL 50 mM $CaCl_2$ and competent bacteria was prepared after overnight incubation at +4˚C. Cationic lipid – pDNA complexes including 4 ng pDNA were mixed with 100 µL competent bacteria, and samples were incubated on ice for 35 min. After incubation, samples on ice were transferred to a 42˚C water bath for 1.5 min and back to ice for 10 min. Samples were mixed with 900 µL LB medium and incubated at 37°C for an hour. After incubation, 100 µL of the transformation mixture were spread over the surface of the agar plate containing 100 µg/mL ampicillin, by $1:10^3$ final dilution. After overnight incubation at 37˚C, transformation efficiencies were calculated.

Preparation of Optimal Lipid–Surfactant-DNA Complexes for Alternative Transformation Method

Optimal lipid – surfactant – DNA complexes were selected according to the results of the gel retardation assay, DNase I protection assay and transformation efficiencies. Complexes were prepared by mixing TE buffer solutions of the pBluescript® plasmid with the cationic lipid DOTAP, zwitterionic neutral helper lipid DOPE (at a ratio of 1:1(w/w), ESCORT™), and zwitterionic surfactant Zwittergent®3-08 (Z-8), at minimum inhibitor concentration (MIC) of 3.75% (w/v).

Alternative Transformation Method

Escherichia coli XL-1 Blue cells and pBluescript®, which contains a gene for ampicillin resistance, were used as a model for the development of an alternative transformation system. A single colony of XL-1 Blue cells was used to inoculate 15 mL of LB medium and grown overnight at 37˚C in a shaking incubator. The 300 µL overnight culture was then subcultured to 15 mL fresh medium and incubated two more hours. 1.5 mL of each culture were then washed with 1 mL 50 mM $CaCl_2$ by centrifugation and the pellets were again suspended with 200 µL 50 mM $CaCl_2$ solution. Naked plasmids and plasmids complexed with cationic lipids and surfactants at various ratios were mixed with prepared bacteria. Samples were incubated either on ice or at 37°C for various time periods (1, 5, 10, 15, 30 and 60 min). After incubation, samples on ice were transferred to a 42˚C water bath for 1.5 min and back to ice for 5 min. Finally, all the samples were washed with phospha-

te buffer (pH 7.4), LB medium or 5% (w/v) BSA solutions. The pellets were suspended in 500 µL LB medium and inoculated on LB agar containing 100 µg/mL ampicillin, either after incubating at 37°C for one hour or directly without incubation. After overnight incubation, the presence of pBluescript® plasmids in transformed colonies were checked by miniprep plasmid purification.

All the experiments were repeated with different types of *E. coli* strains such as XL-1 Blue, ATCC 25922, JM109, K12 and M15.

RESULTS

Plasmid DNA Isolation

The purity of the plasmid DNA was established by UV spectrophotometry (OD_{260nm} / OD_{280nm}=1.8).

Effect of Plasmid:Lipid Ratios on Complex Formation and Transformation Efficiency

Agarose gel electrophoresis images of the plasmid DNA - cationic lipid complex formulations which were prepared at different ratios are shown in Figure 1. Complex formation can be achieved starting from 1:0.125 w/w pDNA:lipid ratios.

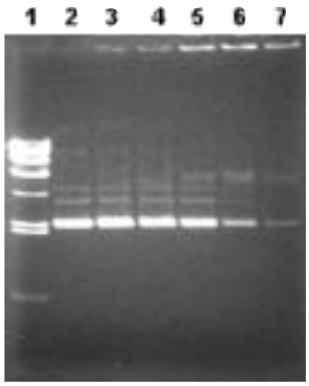

Figure 1. Gel retardation assay.
Lane 1: λ DNA Hind III Digest MW Marker
Lane 2: Plasmid DNA
Lane 3: Plasmid DNA:Cationic Lipid → 1:0.125 w/w
Lane 4: Plasmid DNA:Cationic Lipid → 1:0.25 w/w
Lane 5: Plasmid DNA:Cationic Lipid → 1:0.5 w/w
Lane 6: Plasmid DNA:Cationic Lipid → 1:1 w/w
Lane 7: Plasmid DNA:Cationic Lipid → 1:2 w/w

Plasmid DNA can be protected from DNase I degradation by complexing with lipids. Retardation effect and stabilization from DNase I were directly proportional to the amount of lipids. As seen in Figure 2, complexes were kept stable for 15 min at 37°C, even with minimal lipid ratios.

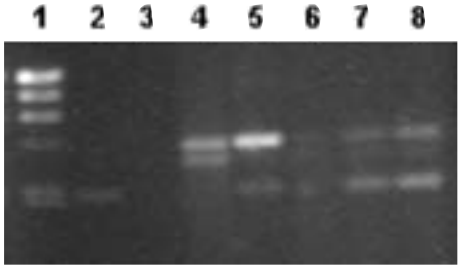

Figure 2. DNase I degradation.
Lane 1: λ DNA Hind III Digest MW Marker
Lane 2: Plasmid DNA
Lane 3: Plasmid DNA*
Lane 4: Plasmid DNA:Cationic Lipid → 1:0.125 w/w*
Lane 5: Plasmid DNA:Cationic Lipid → 1:0.25 w/w*
Lane 6: Plasmid DNA:Cationic Lipid → 1:0.5 w/w*
Lane 7: Plasmid DNA:Cationic Lipid → 1:1 w/w*
Lane 8: Plasmid DNA:Cationic Lipid → 1:2 w/w*
*LANE 3 - 8 : SAMPLES INCUBATED WITH DNASE I

Cell cultures could be transformed at all pDNA:lipid ratios. According to the results given in Table 1, efficiency of transformation increased until the ratio reached 1:0.5 w/w pDNA:lipid ratio, and then decreased rapidly.

Table 1. Transformation efficiencies of plasmid DNA:lipid complexes at various ratios

Plasmid DNA:Lipid Complexes	Transformation efficiency*, no. of transformants X 10^7 per mg of pDNA (cfu/mg)
Plasmid DNA	3.5 ± 0.38
Plasmid DNA: Lipid → 1: 0.125 w/w	3.8 ± 0.42
Plasmid DNA: Lipid → 1: 0.25 w/w	3.4 ± 0.28
Plasmid DNA: Lipid → 1: 0.5 w/w	3.1 ± 0.25
Plasmid DNA: Lipid → 1: 1 w/w	1.9 ± 0.21
Plasmid DNA: Lipid → 1: 2 w/w	1.6 ± 0.18

* Values represent mean ± SEM of the triplicate plates

Effect of Factors on Transformation Efficiency of Alternative Method

Investigated factors such as concentration of cationic lipids, effect of surfactants, freshness of the culture, incubation temperature, incubation time, wash treatment and bacterial strains were found to affect transformation efficiency. Concentrations of cationic lipids and surfactants also played an important role in the transformation. It was determined that the optimum final concentration for the highest transformation efficiency was 1.25% v/v for DOTAP/DOPE and 0.05% w/v for Zwittergent®3-08.

Freshness of the culture was determined to be an important factor for DNA uptake into bacteria. Efficiency of transformation was nearly the same with naked plasmids or plasmids complexed with cationic lipids. With old cultures, lipids showed significant ability to induce transformation. The number of transformants were found dependent upon the temperature. Heat shock (incubation on ice followed by incubation at 42°C) increased the transformation efficiency when compared with constant incubation at 37°C (Fig. 3).

Procedures are also influenced by the incubation period on ice. In our procedure, transformation efficiency increased with time in the first 15 min, then decreased (Fig. 4).

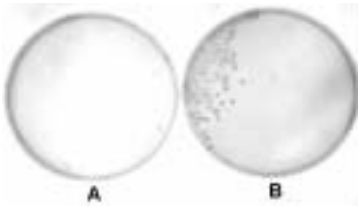

Figure 3. Effect of incubation temperature on transformation efficiency.

Transformation at A :Constant incubation at 37°C;
B : Incubation on ice, followed by 42°C and back to ice.

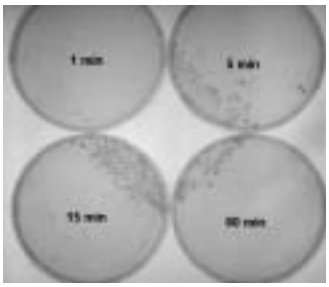

Figure 4: Effect of incubation period on transformation efficiency.

To determine the utility of washing solution on the transformation efficiency, three different solutions were used, and the best results were obtained with 5% (w/v) BSA when compared to LB medium and phosphate buffer (pH 7.4) (Fig. 5).

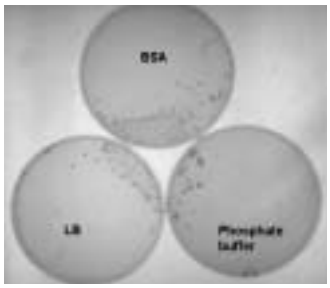

Figure 5. Effect of wash solution on transformation efficiency.

Although all the strains transformed efficiently, the best results were obtained with JM109, XL-1 Blue and M15.

Advantages of the alternative transformation method over classical transformation method can be seen in Table 2.

Table 2. Advantages of alternative transformation method* over classical transformation method.

	Classical Transformation Method	Alternative Transformation Method
For competent bacteria preparation		
Fresh culture medium	is required	is not required
Incubation of bacterial pellet on ice for an hour in $CaCl_2$ solution	is required	is not required
After the step above, one night incubation at 4°C	is required	is not required
For bacterial transformation		
Incubation time required for competent cells and pDNA complexes on ice	35 minutes	15 minutes
1 hour incubation at 37°C before spreading over agar plates	is required	is not required

*** Bacterial Transformation Kit, TPE Patent, No:TR 1999 01199B, 1999.**

DISCUSSION

Complexes between negatively charged DNA and positively charged lipids can be produced simply by electrostatic and hydrophobic interactions. Cationic lipids are well-known materials for transferring DNA into mammalian cells, and cell lines respond differently to the various types of gene delivery systems. In addition to the mammalian cells, there have also been many efforts to optimize and simplify efficiency of bacterial transformation. Here we describe a protocol using cationic lipids, zwitterionic surfactants and washing treatments for the purpose of transforming bacteria.

In this study, stability and efficiency of the complexes were directly related to pDNA:lipid ratios and incubation times, as expected. Even with minimal lipid amounts, plasmids were stable for 15 min. The effects of exogenous lipids are explained by the interactions of membrane transferases with phospholipids[32]. Addition of cationic lipids to pDNA leads to the formation of highly stable condensed structures that protect DNA from nuclease digestion[16].

Transformation efficiency was found dependent on the pDNA:lipid ratio. The highest effect was achieved with 1:0.5 w/w pDNA:lipid ratio; at more than 1:1 pDNA:lipid ratios, the number of transformed bacteria decreased. This effect may involve toxicity of the cationic lipids on the cells as known from transfection studies[16].

Zwitterionic surfactants were also included in preparations in order to regulate electrostatic interactions, decrease toxic effects due to the high positive charge and enhance cell wall permeability[33]. In our previous study[34,35], among the investigated zwitterionic surfactants, Zwittergent®3-08 (Z-8) was selected as the most efficient surfactant in transformation.

The freshness of bacterial culture is another factor in efficient transformation. In the protocol we used lipid-containing formulations which showed a pronounced effect on the transformed cells when the culture was old. This result shows that it is advantageous to store cultured cells for future use.

Temperature shift is an important point for transformation. In the proposed method, 15 minutes of ice treatment was enough for efficient transformation, and an additional incubation step in liquid medium at 37°C was not required.

Washing steps are used in some protocols in the publications. In our procedure washing bacteria with 5% (w/v) BSA solution after the transformation step assured the survival of transformants probably by removing toxic amounts of lipids, detergents and other toxic products originating from bacteria in the medium. Even if the lipids were not included during transformation, this washing procedure positively affected the number of transformants.

Different bacterial strains respond differently to the procedure, and widely used E. coli strains such as JM109, XL-1 Blue and M15 could be transformed efficiently.

As an alternative to the known transformation protocols, we propose an easy, rapid and highly efficient method for bacterial transformation without the competent cell preparation step.

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